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Abstract Grazing frequency, grazing intensity, plant health, and grazer abundances were examined under two different fertilization regimes and compared to unfertilized areas of *Juncus roemerianus* (black needlerush) marsh. These measurements were taken in three separate sites: two low elevation sites and one high elevation site. Fertilizer was added over one grazing season (April–August) with measurements taken over the same time period at each site. In general, grazing frequency and intensity was higher in the fertilized treatments than unfertilized treatment; however, plant health was similar among treatments. Relative grazer abundance was higher in the fertilized treatments than unfertilized. From these results, it can be suggested that black needlerush grazers preferentially congregate and graze in areas of marsh with higher nutrient content in the leaves. However, the effects of this increased grazing on plant health (leaf lengths, biomass, and percent living biomass) were small after one grazing season. This information furthers our understanding of the potential effects of anthropogenic eutrophication on salt marshes and can be used by managers focusing on conservation and restoration of black needlerush.

Keywords Anthropogenic · Eutrophication · Nitrogen · *Juncus roemerianus* · Black needlerush

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Introduction

Anthropogenic nutrient loading may affect coastal systems in a variety of ways, with impacts ranging from negative to neutral to positive (Anton et al. 2011; Deegan et al. 2012; Duarte 1995; Heck et al. 2006). Recent research shows that nutrient enrichment may stimulate the intensity of herbivory on marsh plants, thereby affecting the extent of top-down regulation of marsh primary productivity by herbivores (Denno et al. 2002; Finke and Denno 2004; Gratton and Denno 2003; Silliman and Bortolus 2003). Positive relationships between fertilization and grazing rates have been found within *Spartina alterniflora* (smooth cordgrass)-dominated marshes (Murphy et al. 2012). Sala et al. (2008) found decreases in plant biomass of 45 % when insect consumers (grasshoppers and leaf hoppers) were allowed access to fertilized plots of smooth cordgrass. The intensity of herbivory on fertilized plots was much higher than on non-fertilized plots, suggesting a great role of herbivores as controls of primary productivity under fertilized conditions.

Most research on marsh grazing has been performed in the smooth cordgrass dominated-salt marshes on the East Coast of the USA (Jefferies 1997; Johnson and Jessen 2008; Sala et al. 2008; Silliman and Zieman 2001). Conversely, few marsh grazing studies have been conducted in Gulf of Mexico marshes where *Juncus roemerianus* (black needlerush) is one of the most prevalent salt marsh plants (Eleuterius 1976). The abundance of black needlerush makes it a primary component to many conservation and restoration efforts along the Gulf of Mexico coast. To help guide these efforts and improve our understanding of the ecology of these important systems, studies on the interactions between herbivory and nutrient enrichment in black needlerush marshes are needed.

Black needlerush contains amino acids that are important to the survival and flight of grasshoppers (de la Cruz and

Gabriel 1974). Grasshoppers are responsible for the majority of grazing on black needlerush in the Gulf of Mexico (Wason and Pennings 2008). In their juvenile stage, grasshoppers are known to target plants containing high concentrations of amino acids, which are rich in nitrogen (Parsons and de la Cruz 1980). In addition, grasshoppers tend to focus their grazing efforts along the top section of black needlerush leaves, which have higher amino acid concentrations than the mid and basal leaf sections (Parsons and de la Cruz 1980). These findings show that grasshoppers seek and select leaf tissue with higher nutritional quality (i.e., higher nitrogen content) while foraging on black needlerush. Anthropogenic nitrogen inputs into marshes can lead to higher nitrogen contents in the leaves of marsh plants (Hunter et al. 2008, 2014; McFarlin et al. 2008; Murphy et al. 2012; Sparks et al. 2013). Therefore, human-induced nitrogen enrichment of black needlerush marshes could significantly alter the dynamics of grasshopper herbivory and lead to higher levels of consumption in fertilized marshes in relation to non-fertilized marshes. In turn, intense herbivory may significantly control primary productivity in these marshes (Cebrian 1999; Finke and Denno 2004; Silliman and Bortolus 2003).

Recent observations along the northern Gulf of Mexico (nGOM) coast have reported intense grazing on black needlerush (Carrier 2013), which is in stark opposition to earlier reports of low herbivory (Hunter et al. 2014; Parsons and de la Cruz 1980). Furthermore, intense grazing on black needlerush leaves may increase leaf senescence and breakage, as it has been shown for other marsh plants (Alberti et al. 2011). Therefore, intense grazing could accelerate the flow of black needlerush leaf biomass into the detrital pool, along with exerting significant control on primary productivity (Cebrian 1999).

In this study, we examine how the intensity of herbivory by grasshoppers on black needlerush leaves varies across a gradient of fertilization. We assess whether herbivory increases with higher fertilization and whether increased herbivory results in depressed plant performance. The results, from this study, further our understanding of how anthropogenic nutrient inputs may affect the ecology of black needlerush marshes, and provide valuable information that can help guide conservation and restoration efforts for these marshes.

Methods

Study Sites and Experimental Setup

The study sites were located within the Grand Bay National Estuarine Research Reserve (GBNERR) in Mississippi, USA. We had three sites, two at low elevation (sites 10L and 11L)

and one at relatively higher elevation (site 11H). We determined elevation using Real Time Kinematic (RTK) satellite navigation with a Trimble R8-Model 3 base and rover RTK system. The low elevation sites were ≈0.1 m above MLLW and approximately 10 m inland from Bayou Heron, the primary water body in the area, and the high elevation site was ≈0.25 m above MLLW and approximately 200 m inland from Bayou Heron (Fig. 1). Site 10L was sampled monthly from April to August 2010, whereas sites 11L and 11H were sampled every other month from April to August 2011. This sampling timing was chosen to encompass the annual cycle of growth and biomass peak of herbivorous grasshoppers (Carrier 2013; Davis and Gray 1966; Smalley 1960; Stout 1984).

Each site contained five randomized blocks with three treatments (Fig. 1). The treatments were no fertilization (NO), moderate fertilization (M), and high fertilization (H). One plot of each treatment was randomly placed within each block. Plots and blocks were spaced 10 m apart from each other to avoid cross-fertilization. The dimensions of each plot were 2 m×2 m and marked with PVC poles on the corners. Fertilization occurred biweekly by hand-casting fertilizer with a 16:1 nitrogen (N) to phosphorus (P) ratio throughout each fertilized plot. This ratio was used since it closely resembles the N to P ratio of black needlerush leaves (Hunter et al. 2014). Fertilization began 1 month prior to the first sampling (March) and continued throughout the experiment (end of August) at a rate of 242 g N m^{-2} year^{-1} (15 g P m^{-2} year^{-1}) for high fertilization and 121 g N m^{-2} year^{-1} (7.5 g P m^{-2} year^{-1}) for moderate fertilization. These fertilization levels reflect realistic anthropogenic nutrient loads into marshes while not being high enough to cause ammonium toxicity (Levine et al. 1998; Valiela et al. 2000, 2001).

Measurements

Plant samples were taken at the end of each month from April to August in 2010 at site 10L and the end of every other month from April to August in 2011 at sites 11L and 11H. Two 15 cm×15 cm quadrats were randomly tossed within each plot at every sampling date. All vegetation within the quadrat was clipped at the sediment surface and, once back at the lab, frozen until further processing. Leaves were then thawed and measured for the length of each clipped leaf and the percentage of leaves grazed. For each leaf, we also measured a number of metrics indicative of herbivory intensity including the following: number of grazing scars, length of longest grazing scar, leaf grazing degree, and leaf breakage probability. The number of grazing scars was expressed as number per meter of leaf length, and the length of the longest scar was expressed as percentage of leaf length. Leaf grazing degree was an arbitrary, qualitative ranking system of the extent of herbivory on the leaf, which varied from 0 (lowest) to 5

Fig. 1 Schematic of experimental layout. Treatment arrangement was randomized for each block
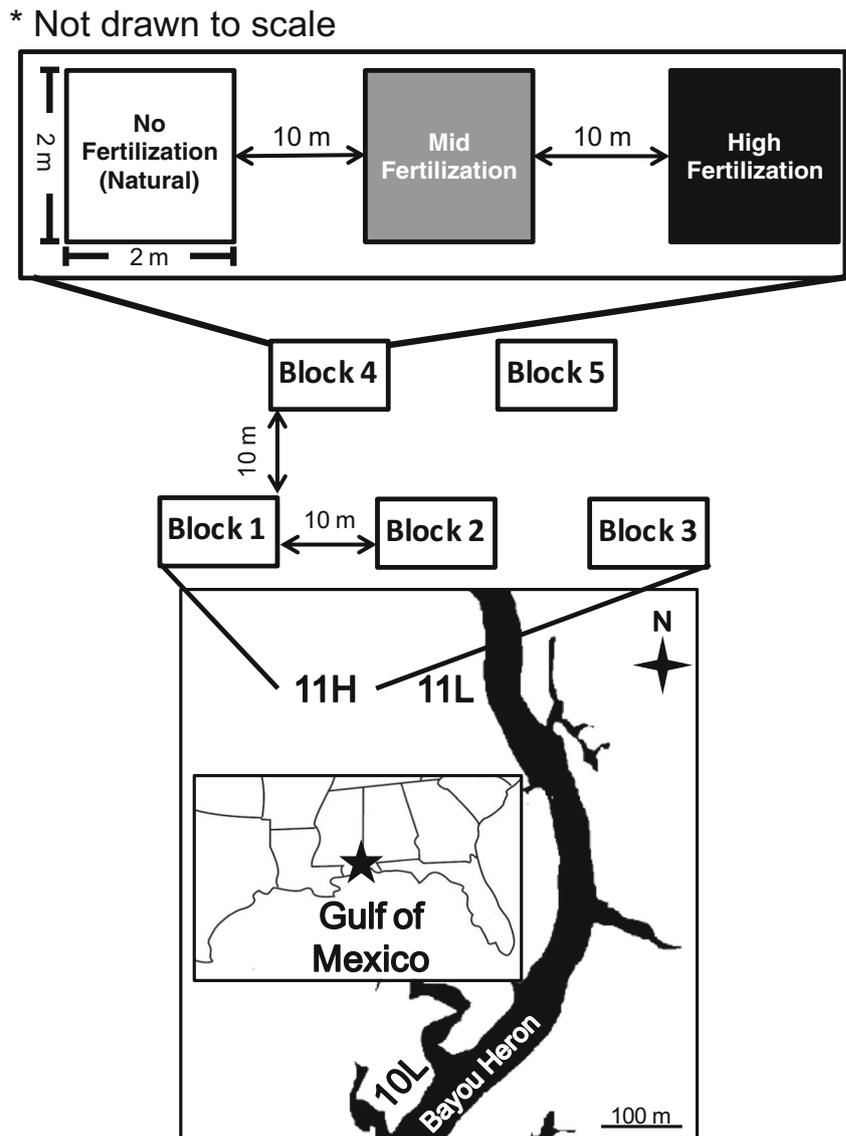
(highest) (Fig. 2). Values of 0 corresponded to no grazing on the leaf. Values of 1 approximately corresponded to 0.1–1 % of leaf area grazed and values of 5 corresponded to >30 % of leaf area grazed, with intermediate values being equidistantly distributed between these two extremes (Fig. 2). Leaf breakage probability was also an arbitrary ranking system reflecting how likely it was for the leaf to break off as a result of herbivory (Fig. 3). Values were also scored from 0 (lowest) to 5 (highest). To score these leaves, the most severe (i.e., deepest and most likely to break) scar on each leaf was identified and assigned a breakage probability. Values of 0 corresponded to no grazing on the leaf, and hence a nil breakage probability. Values of 1 corresponded to small grazing scars with only surface damage and values of 5 to the loss of a tip due to grazing (Fig. 3). Intermediate values reflected intermediate stages between these two extremes. The same individual (ES) made all these measurements to ensure

consistency. For all grazing metrics, only grazing scars from the current grazing season were counted. These scars were determined to be from the current grazing season by the presence of frayed plant material around the scar. After taking all the measurements, a subsample of three green (no senesced parts) leaves was taken from each quadrat for N content analysis on a Costech ECS 410 CHNSO Analyzer. Leaves were then further separated into living and senesced parts. The samples were dried and weighed with green, dead and total biomass calculated for each quadrat.

In 2011, one insect trap was deployed in each plot at sites 11L and 11H to quantify relative grasshopper abundance across treatments. The traps consisted of a clear 30 cm× 30 cm plexiglass plate mounted on a PVC pole at a 45° angle. The center of the plexiglass plate sat at approximately three fourths of the maximum canopy height since most grazing occurs on the top part of the leaves. The plexiglass plates were
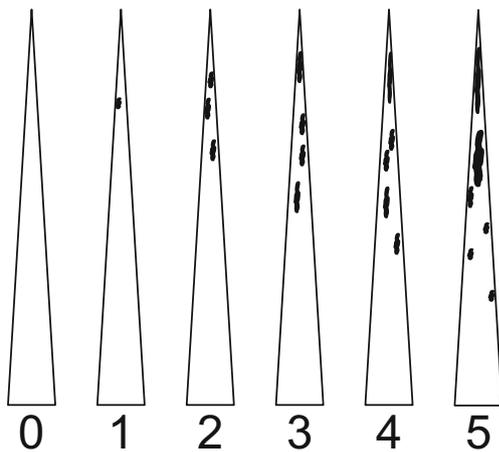
Fig. 2 Ranking system for whole leaf marsh grazing degree with *0* representing no grazing, *1* being a low degree of grazing, and *5* being a high degree of grazing

coated with Tanglefoot, a non-attractive sticky compound. After 4 days of deployment, the traps were revisited with insects counted and picked off the traps. The traps were then scraped clean with a paint scraper followed by a new application of Tanglefoot. Each insect trap was sampled four times per month. CPUE (i.e., catch per unit effort, individuals per trap per day) was calculated for each plot for every sampling interval.

Statistical Analysis

The average of leaf length, leaf nitrogen content, metrics of herbivory intensity, and biomass values were calculated for each quadrat. The averages of the two quadrats from the same plot were averaged from each sampling time. All four grasshopper abundance samples within the same month were averaged for each plot. Those averages were used as our replicates in the statistical analysis. We first ran a repeated measures ANOVA (RMANOVA) with block, fertilization (among-subject factor), and time (within-subject factor) for each site and variable. Block was never significant, thus we pooled all
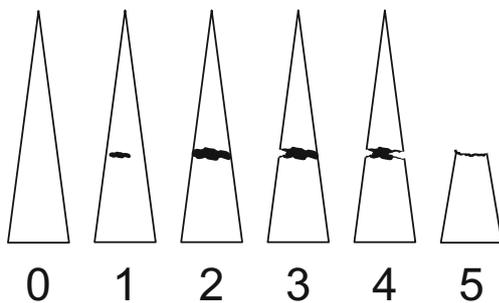
Fig. 3 Ranking system for breakage probability of individual grazing scars with *0* representing no grazing, *1* being the lowest probability of breaking, and *5* being a broken leaf

blocks together ($n=5$) and reran the balanced RMANOVA with only fertilization ($n=3$) and time ($n=4$ for 2010; $n=2$ for 2011) to increase the chance of finding significant effects by these factors (Quinn and Keough 2002). No grazing occurred during April in each of the 2 years; thus, it was excluded from the analysis. If treatment was significant, but time and the interaction between treatment and time were not, dates were pooled for post hoc Tukey comparisons among treatments. If a significant interaction between treatment and time occurred, or treatment and time were both significant but the interaction was not, Tukey comparisons were done on each date separately. Post hoc Tukey tests were not conducted when there was a combination of no significant treatment effect and no interaction between treatment and time. Additionally, we compared the maximum cumulative herbivory in August among treatments ($n=3$) and sites ($n=3$) using a two-way ANOVA. If treatment or site was significant, but the other factor and the interaction were not, the insignificant factor was pooled for post hoc Tukey comparisons. If a significant interaction between treatment and site occurred, or treatment and site were both significant but the interaction was not, Tukey comparisons for treatment were done at each site separately with site comparisons done at each fertilization level separately. For the two-way ANOVA, post hoc Tukey tests were not conducted when treatment, site, and the interaction of treatment and site were not statistically significant. Statistical tests were done with R (version 3.0.2) and Sigmastat (version 3.5); significance for all tests was considered at $p<0.05$.

Results

For all sites, most of the measured metrics showed a significant effect of time (Table 1). Nitrogen content and most grazing metrics increased through time (Figs. 4, 5, and 6). Plant metrics (i.e., leaf length, aboveground biomass, and percentage of living biomass) did not show as strong of a time effect as the other metrics (Fig. 7). Grasshopper abundance was also variable through time with site 11H, showing no time effect and grasshopper abundance in site 11L decreasing significantly through time (Fig. 8). Visual observations of grasshoppers body size through time showed an opposite trend to abundance with smaller individuals in May and larger in August. These results are not surprising due to the cumulative nature of grasshopper grazing on black needlerush and seasonality of plant growth and insect abundance; therefore, the rest of the results focus on fertilization effects.

There was a significant interaction between fertilization and time for leaf tissue nitrogen content (Table 1). Because of this interaction, treatments were compared on individual dates with post hoc Tukey tests. In all three, sites there were no differences among treatments at the April sampling ($p>0.592$; Fig. 4). At the June sampling, leaves in the H treatment from

Table 1 Results of RMANOVA for treatment, time, and interaction of treatment×time for all measured response variables

Response variable	Site	Treatment		Time		Treatment×time	
		F value	p value	F value	p value	F value	p value
N content (%)	10L	10.813	*0.002*	12.222	*<0.001*	3.846	*0.015*
	11L	17.255	*<0.001*	17.826	*<0.001*	8.517	*<0.001*
	11H	22.328	*<0.001*	13.549	*<0.001*	4.387	*0.008*
Leaves grazed (%)	10L	7.911	*0.006*	206.602	*<0.001*	0.510	0.797
	11L	0.803	0.471	46.465	*<0.001*	0.578	0.576
	11H	1.525	0.257	42.560	*<0.001*	0.060	0.942
Scars (m^{-1})	10L	0.413	0.670	7.889	*<0.001*	0.703	0.649
	11L	0.347	0.714	0.776	0.396	0.911	0.428
	11H	0.367	0.701	38.570	*<0.001*	0.425	0.663
Longest scar	10L	6.427	*0.013*	41.340	*<0.001*	1.262	0.299
	11L	9.954	*0.003*	102.898	*<0.001*	6.112	*0.015*
	11H	0.661	0.534	30.159	*<0.001*	3.852	*0.049*
Leaf grazing degree	10L	8.105	*0.006*	440.317	*<0.001*	2.191	0.189
	11L	2.198	0.154	89.853	*<0.001*	3.798	*0.050*
	11H	4.361	*0.038*	119.149	*<0.001*	1.824	0.203
Breakage probability	10L	11.544	*0.002*	45.825	*<0.001*	2.553	*0.037*
	11L	0.509	0.613	6.391	*0.027*	0.017	0.983
	11H	5.681	*0.018*	0.258	0.621	0.776	0.482
Leaf length	10L	0.877	0.441	3.529	*0.013*	1.007	0.443
	11L	1.030	0.386	0.561	0.578	1.088	0.385
	11H	1.034	0.385	16.271	*<0.001*	1.934	0.137
Biomass	10L	0.430	0.660	14.317	*<0.001*	0.966	0.474
	11L	0.236	0.794	0.909	0.416	1.671	0.189
	11H	0.813	0.467	1.936	0.166	1.842	0.154
Living biomass (%)	10L	2.676	0.109	9.170	*<0.001*	2.316	*0.035*
	11L	2.503	0.123	4.855	*0.017*	1.283	0.304
	11H	4.988	*0.027*	9.088	*0.001*	0.843	0.512
Hopper CPUE	10L	NA	NA	NA	NA	NA	NA
	11L	3.522	*0.042*	10.590	*<0.001*	1.549	0.197
	11H	3.304	*0.049*	0.753	0.529	0.981	0.455

Italicized *p* values indicate significance (*p<0.05*)

the low elevation sites (10L and 11L) had higher nitrogen content than the NO treatment (Fig. 4a, b). Both fertilized treatments in the high elevation site (11H) had higher nitrogen content in their leaves than the NO treatment at the June sampling (Fig. 4c). At the August sampling, the H and M treatments had significantly higher nitrogen content than the NO treatment at the low elevation sites (Fig. 4a, b) and leaf nitrogen content significantly increased from the NO to M to H treatments at the high elevation site (Fig. 4c).

The only site where fertilized treatments had a higher percentage of grazed leaves across time was 10L (Table 1; Fig. 5a–c). Since both treatment and time showed significance with the RMANOVA (Table 1), we compared treatments at site 10L, on each sampling time separately, and found that the fertilized treatments had similar percentages of leaves grazed and higher than the NO treatment in June and August

(Fig. 5a). When only analyzing the end of the grazing season (August), across all sites, we found that both treatment and site were significant factors (Table 2). Therefore, two sets of pairwise Tukey tests were performed. One set of pairwise Tukey tests compared treatments at individual sites (10L, 11L, and 11H) and the other set compared sites across individual fertilization levels (treatments: H, M, and NO). Treatments compared across sites and sites compared across treatments showed similar percentages of grazed leaves.

When analyzing sites individually (Table 1) or together at the end of the grazing season (Table 2; Fig. 5d–f), the number of grazing scars per meter of leaf was not affected by fertilization. Scars per leaf meter were also found to be similar among sites in August (Table 2).

Comparisons among treatments within sites yielded scar length increasing with fertilization in 10L. However, since
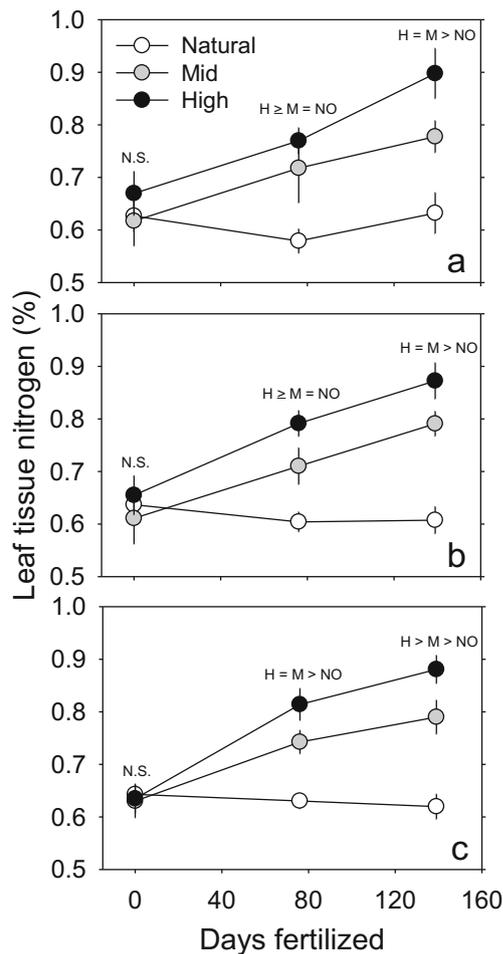
Fig. 4 Percent nitrogen content in black needlerush leaves through time across three fertilization regimes. *Open circles* represent the unfertilized (natural) treatment, *gray circles* represent the mid level fertilization treatment, and *black circles* represent the high level fertilization treatment. *Panels* represent sites **a** 10L, **b** 11L, and **c** 11H. *Lettering above individual sampling dates* indicates results of pairwise comparisons with post hoc Tukey tests within that date. *N.S.* corresponds to no significant difference among treatments, *NO* natural treatment, *M* mid treatment, and *H* high treatment. *Error bars* indicate±1 SE

treatment and time were significant factors (Table 1), comparisons of treatments on individual sampling dates were conducted. We found that the H treatment had significantly longer scars than the NO treatment in July, whereas the H treatment had longer scars than both the M and NO treatments in August (Fig. 5g). Sites 11L and 11H had significant interactions (treatment×time); therefore, comparisons among treatments were done separately for each date. There were no treatment effects in the June sampling for either site; however, both sites displayed treatment effects in August. At the August sampling in site 11L, scar length in fertilized treatments was higher than the NO treatment, but it did not differ between the two fertilized treatments (Fig. 5h). In site 11H, the H treatment had longer scars than the NO treatment (Fig. 5i). There was a significant interaction when comparing site and treatment

in August (Table 2); therefore, pairwise comparisons were conducted. Treatment differences were found at site 10L (H>M=N) and 11L (H=M>NO). Within all treatments, site 10L had longer scars than the other sites ($p<0.019$). Site 11L had longer scars than site 11H when fertilizer was added and had similar scars of similar length without fertilization ($p=0.221$).

For leaf grazing degree, all sites were analyzed for treatment effects on individual sampling dates because treatment and time were significant factors (10L and 11H) or there was a significant interaction between treatment and time (11L; Table 1). For site 10L, the fertilized treatments had higher leaf grazing degrees than the NO treatment in June and August, while the only difference in July was the H treatment having a higher grazing degree than the NO treatment (Fig. 6a). There was no treatment effect in June for sites 11L and 11H; however, in August, both sites showed higher grazing degrees in the fertilized treatments than the NO treatment (Fig. 6b, c). For August comparisons across sites, both treatment and site were significant factors (Table 2). Pairwise Tukey results show significant differences among treatments within all 3 sites (H=M>NO). Within fertilized treatments, site 10L had higher leaf grazing degrees than 11H while there were no site differences within the NO treatment.

Site 10L had a significant interaction (treatment×time) for breakage probability (Table 1); therefore, treatments were compared on each date separately with significant treatment effects in May (H≥M=NO) and August (H>M>NO; Fig. 6d). In site 11L, breakage probability increased with time but did not with treatment (Table 1; Fig. 6e). In site 11H, only treatment was significant and, thus, we pooled all dates for multiple comparisons across treatment levels. At this site, the H treatment showed a higher breakage probability than the NO treatment, while the H treatment was equivalent to the M treatment which was equivalent to the NO treatment (Fig. 6f). There was a significant interaction when comparing breakage probability across sites and treatments in August (Table 2). Treatment differences were found at site 10L (H>M>N) and 11H (H≥M=NO). Significant differences among sites within fertilization treatments H (10L>11L=11H), M (10L= 11L>11H), and NO (10L=11L>11H) were also observed.

Leaf lengths across all sites showed no effect of treatment (Table 1; Fig. 7a–c). When only analyzing the August sampling, we found no significant differences across treatments or sites (Table 2).

Overall leaf biomass showed similar trends to leaf length with no treatment effect in any of the sites through time (Table 1; Fig. 7d–f). In August, there was no treatment effect, whereas there was a site effect (Table 2). When pooling the treatments for post hoc comparisons across sites, we found that site 10L had higher biomass than sites 11L and 11H (Fig. 7d–f).

In site 10L, there was a significant interaction between treatment and time with regards to the percentage of living
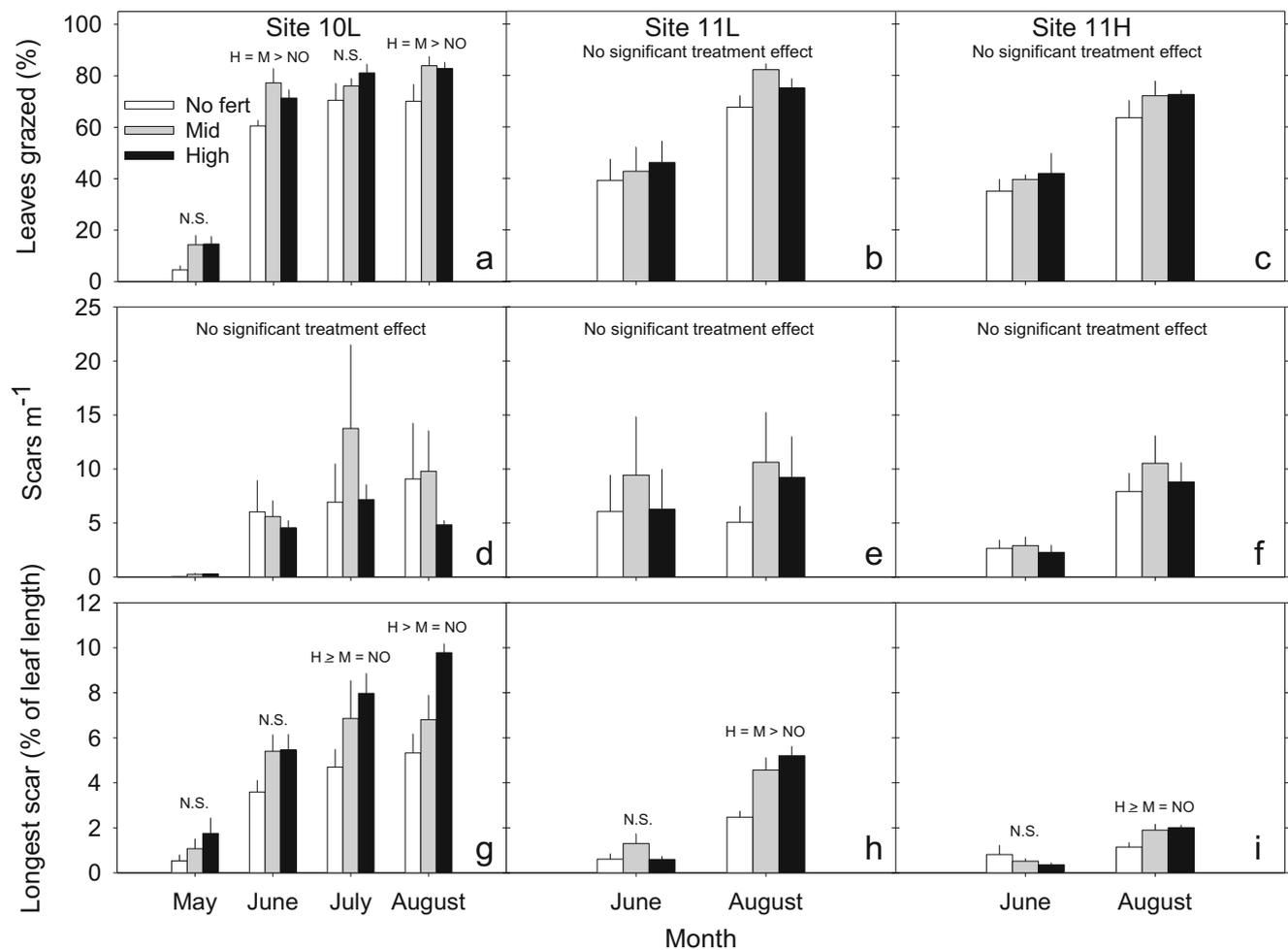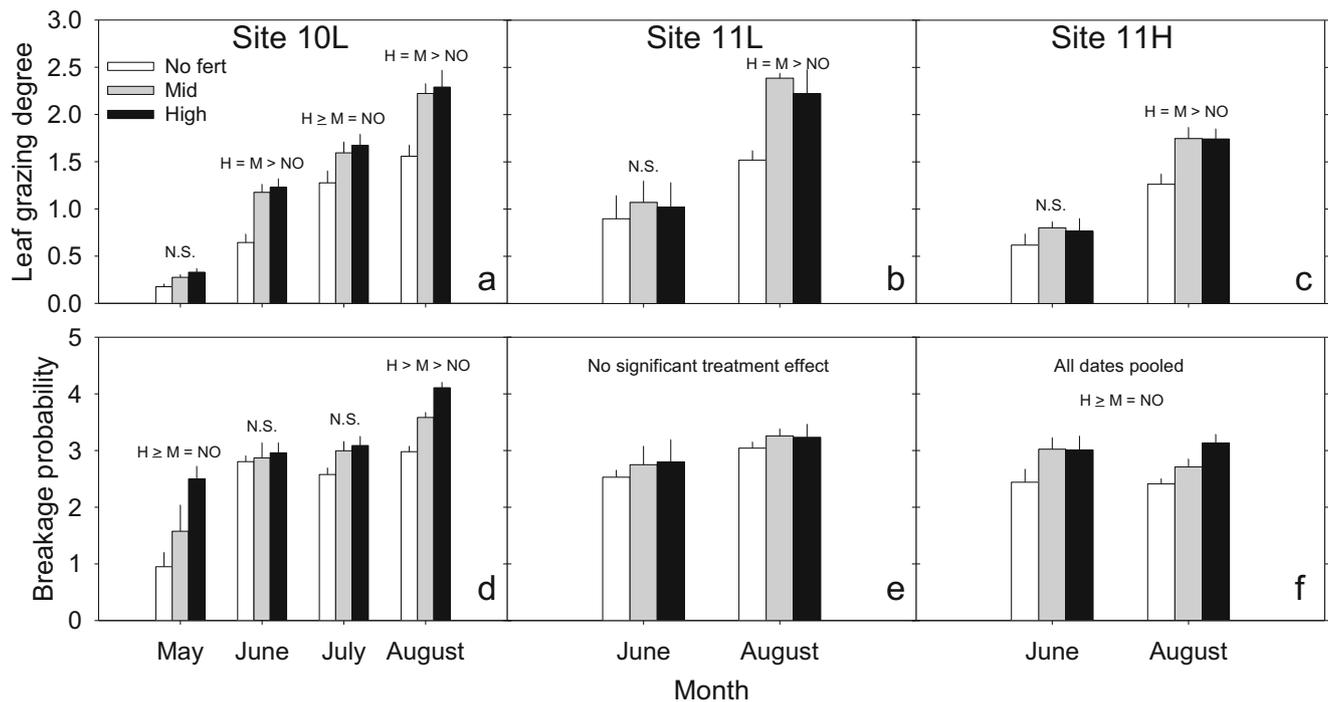
Fig. 5 Grazing metrics through time for the natural, mid, and high fertilization treatments. Grazing metrics include **a**, **b**, **c** percentage of leaves grazed, **d**, **e**, **f** scars per meter of leaf tissue, and **g**, **h**, **i** longest scar as a function of percentage leaf length. *Panel columns* represent study sites with column **a** representing site 10L, **b** representing site 11L, and **c** representing site 11H. *Lettering above individual sampling dates*

indicates results of pairwise comparisons with post hoc Tukey tests within that date. *N.S.* corresponds to no significant difference among treatments, *NO* natural treatment, *M* mid treatment, and *H* high treatment. No pairwise comparisons were conducted when treatment was insignificant. *Error bars* indicate ±1 SE

biomass (Table 1); therefore, each month was analyzed separately for treatment differences. In June and August, we found differences among treatments with the percentage of living biomass being higher in the H treatment than the other treatments in June and higher in the fertilized treatments than the NO treatment in August (Fig. 7g). Site 11L showed no treatment effect (Table 1; Fig. 7h). There was not a significant interaction between treatment and time in site 11H, but both treatment and time were significant. Thus, we compared treatments for each date separately. August was the only month that had a treatment effect with the H treatment having a higher percentage of living biomass than the NO treatment (Fig. 7i). When analyzing across sites at the end of the grazing season (August), the percentage of living biomass was significantly different among treatments and sites (Table 2). Within site 11L, the fertilized treatments had a higher percentage of living biomass than the NO treatment, whereas the only

significant difference within site 11H was the H treatment having higher values than the NO treatment. Sites were similar for percentage of living biomass when compared within individual fertilization levels.

Since treatment and date were significant factors for relative grasshopper abundance in site 11L (Table 1), treatment effects were analyzed for individual dates. The only difference we found was in May with the H treatment having more trapped grasshoppers than the NO treatment; however, the H treatment was equivalent to the M treatment which was equivalent to the NO treatment (Fig. 8a). Only treatment was significant in site 11H; therefore, dates were pooled for analysis. This pooled data showed more grasshoppers in H treatment than the NO treatment (Fig. 8b). When comparing treatment and site effects in August, neither site ($p=0.660$) or treatment ($p=0.622$) were significant.

Fig. 6 Ranked grazing metrics through time for the natural, mid, and high fertilization treatments. Grazing metrics include **a**, **b**, **c** total leaf grazing degree and **d**, **e**, **f** breakage probability of individual scars. *Panel columns* represent study sites with column **a** representing site 10L, **b** representing site 11L, and **c** representing site 11H. *Lettering above a specific sampling date* represents a pairwise comparison within that date while lettering centered in the plot represents comparisons pooled across date. *N.S.* corresponds to no significant difference among treatments, *NO* natural treatment, *M* mid treatment, and *H* high treatment. No pairwise comparisons were conducted when treatment was insignificant. *Error bars* indicate±1 SE

Discussion

To our knowledge, this study is the first to test the effect of different fertilization levels on marsh grasshopper herbivory and its subsequent effect on the plants in a black needlerush marsh. Results from this study led to four principal findings: (1) grazing frequency and intensity was highest in fertilized areas, (2) the effect of increased grazing frequency and intensity had little to no effect on plant health and biomass at the end of one grazing season, (3) marsh grasshoppers congregated in the fertilized areas, and (4) temporal and spatial dynamics can influence marsh grasshopper grazing and effects on black needlerush.

Fertilization has been shown to increase grazing in smooth cordgrass-dominated salt marshes (Murphy et al. 2012; Sala et al. 2008); however, only one study could be found that investigated the relationship between fertilization and grazing intensity in black needlerush dominated salt marshes. Hunter et al. (2014) did not find increases in grazing with fertilization; however, their experiments were performed in the marshes of NW Florida, where grasshopper grazing and abundance is lower than the GBNERR (Carrier 2013).

Marsh grasshoppers are known to primarily feed on individual leaf sections that are of higher nutritional quality (Parsons and de la Cruz 1980); this ability to select leaf sections with higher nutritional quality suggests these grazers would also preferentially graze within fertilized areas of natural marsh. For this reason, we expected grazing frequency and intensity to increase as the degree of fertilization increased. We observed increased leaf tissue nitrogen concentrations with increased levels of fertilization (Fig. 4); therefore, fertilization load can be suggested as a cause for any observed differences in grazing and effects of grazing among treatments.

The majority of the grazing frequency and intensity metrics we measured showed that fertilized areas were more heavily grazed than unfertilized areas, either over the entire duration of measurements or by the end of the grazing season (August). These results confirm marsh grasshoppers exert higher grazing pressure in fertilized areas than non-fertilized areas. The only grazing frequency and intensity metric measured that did not show higher values in the fertilized areas was the scars per meter of leaf. However, the scars present were both longer and more severe (breakage probability) in the fertilized treatments (Figs. 5 and 6). Therefore, the overall grazing per leaf was higher in the fertilized areas, and this is represented by the overall leaf grazing degree (Fig. 6). Interestingly, higher grazing pressure was achieved through fertilizing within previously documented eutrophication levels (Levine et al. 1998; Valiela et al. 2000, 2001) for only one grasshopper season (April–August); therefore, marsh hoppers can quickly
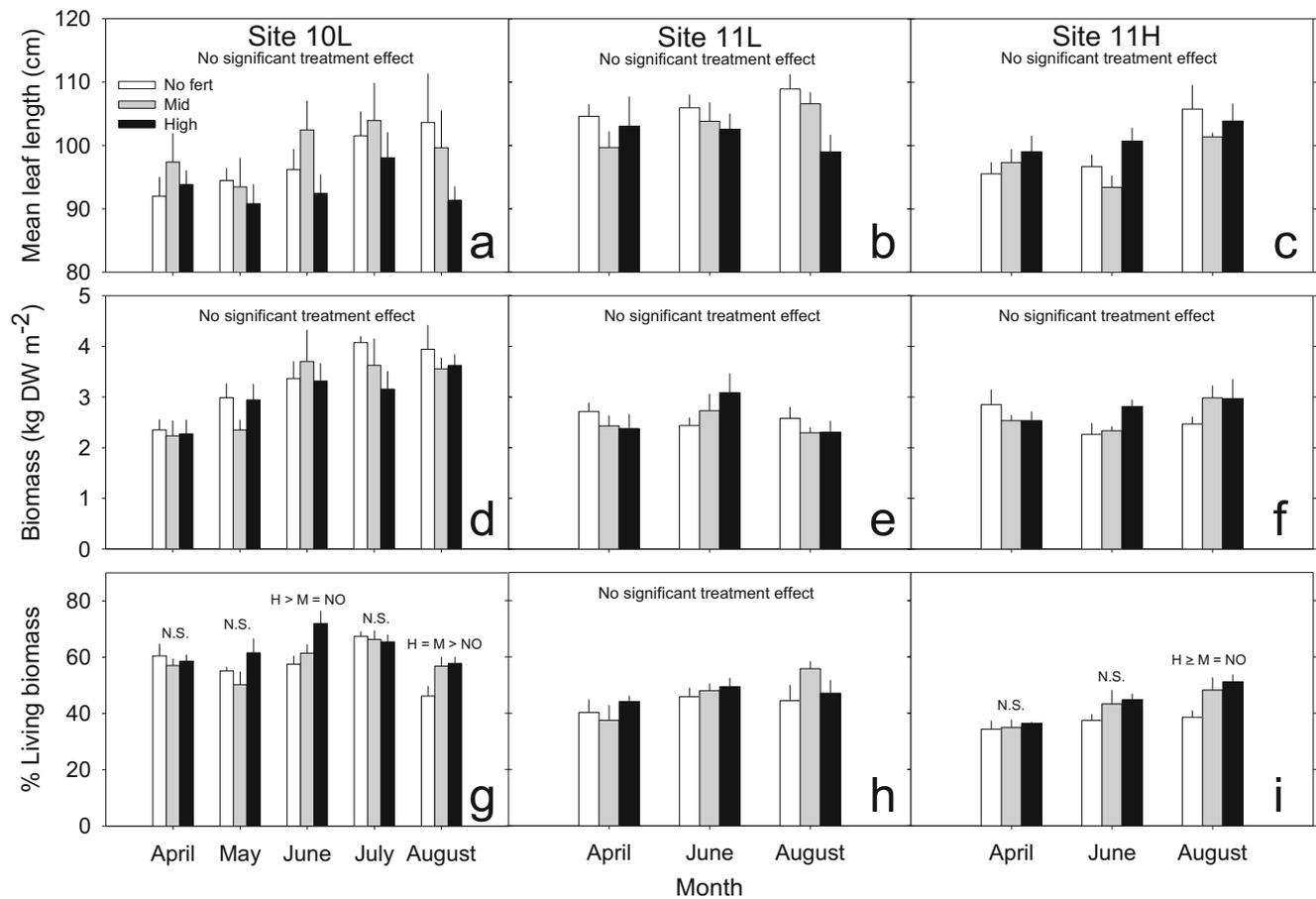
Fig. 7 Plant health metrics through time for the natural, mid, and high fertilization treatments. Measured plant health metrics include **a**, **b**, **c** mean leaf length, **d**, **e**, **f** aboveground biomass, and **g**, **h**, **i** percentage of living aboveground biomass. *Panel columns* represent study sites with column **a** representing site 10L, **b** representing site 11L, and **c** representing site 11H. *Lettering above individual sampling dates* indicates results of pairwise comparisons with post hoc Tukey tests within that date. *N.S.* corresponds to no significant difference among treatments, *NO* natural treatment, *M* mid treatment, and *H* high treatment. No pairwise comparisons were conducted when treatment was insignificant. *Error bars* indicate ±1 SE

determine areas with higher N content and migrate to them. If fertilization occurred over multiple years, nutrient content in the leaves should continue to increase (Hunter et al. 2014), in turn, fuelling more grazing.

While we found increases in grazing frequency and intensity for fertilized areas, we found no drastic decreases in any of the measured plant health metrics (leaf lengths, biomass, and percent living biomass); however, we found some suggestive effects. Overall mean leaf lengths and biomass were lowest in the highly fertilized areas, albeit not significant, which is likely caused by the removal of leaf tips from heavy grazing. Complementary information to the leaf tip removal theory is found when analyzing the percentage of living biomass. Overall, there was a higher percentage of living biomass in the fertilized than unfertilized treatments. Black needlerush leaves senesce from the tip down; thereby, the loss of tips through grazing would increase the proportion of living aboveground biomass and accelerate the pathway of standing biomass to the detrital pool. In marsh periwinkle and smooth

cordgrass grazing studies on the US east coast, there were large reductions in plant health metrics with increased grazing (Sala et al. 2008). A possible explanation for these results is the slow growth and morphology of black needlerush relative to smooth cordgrass (Cebrian and Duarte 1994). Black needlerush is a slow growing plant that assimilates nutrients at a much slower pace than other marsh plants (Hunter et al. 2014). Therefore, more time is needed to achieve drastic differences in N content in fertilized areas vs. non-fertilized areas. Black needlerush leaves are round and rigid, formed primarily of lignin with a thin skin of nutrient rich photosynthetic material (Eleuterius 1975). Grasshoppers primarily consume the thin photosynthetic skin of black needlerush (Parsons and de la Cruz 1980); therefore, the ratio of palatable mass to leaf mass is small in black needlerush. Alternatively, smooth cordgrass leaves are faster growing than black needlerush leaves and have a flat morphology (Stout 1978). Faster growth allows smooth cordgrass to assimilate more nitrogen from a fertilization event into its leaves on shorter
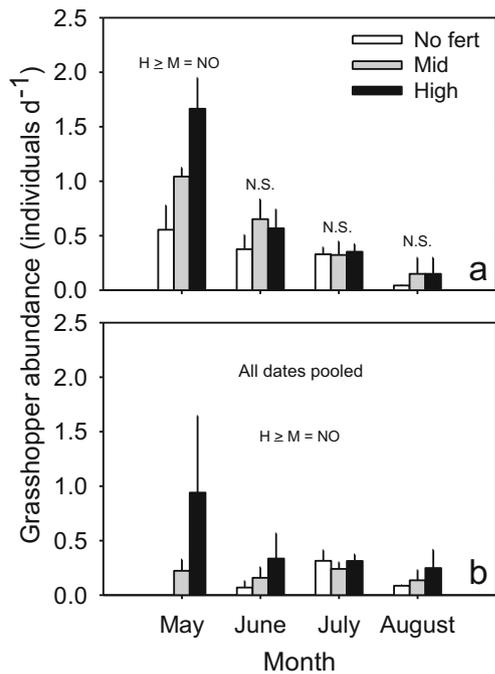
Fig. 8 Relative grasshopper abundances through time for the natural, mid, and high fertilization treatments. Panels represent sites **a** 11L and **b** 11H. *Lettering above a specific sampling date* represents a pairwise comparison within that date while lettering centered in the plot represents comparisons pooled across date. *NO* natural treatment, *M* mid treatment, and *H* high treatment. *Error bars* indicate ±1 SE

time scales (Hunter et al. 2008, 2014). Thin and smooth cordgrass leaf morphology is likely more prone to grazer damage since there is a higher ratio of palatable mass to leaf mass (Renaud et al. 1990); therefore, more of each individual leaf can be consumed in smooth cordgrass relative to black needlerush.

It is important to note that all of these observed differences between fertilized and non-fertilized areas, with regard to grazing and plant health, occurred after only fertilizing for one grasshopper-growing season. It is possible that continual

fertilization, as seen in anthropogenically influenced systems (Howarth et al. 2002), could induce increased grazing over multiple consecutive grazing seasons. This persistent grazing pressure could lead to decreases in plant health (Deegan et al. 2012; Sala et al. 2008) and/or changes in energy flow through accelerating detrital production. Therefore, long-term fertilization studies are critical to understand the effects of persistent eutrophication on plant health and grazing dynamics.

The greatest marsh grasshopper abundances occurred in the fertilized treatments, predominately during the month of May. Visual observations of these grasshoppers suggest that they were recent hatchlings due to their small size. Grasshopper size increased through time, while abundances decreased, supporting findings from previous studies that found similar trends (Smalley 1960). The trend of more grazers in fertilized treatments persisted through the experiment. However, the differences among treatments did diminish as time progressed. The diminishing differences among treatments through time for grasshopper abundances is likely a function of mortality but could also be a consequence of grasshoppers developing territorial behavior as they mature, thereby hindering the ability of grasshoppers to congregate in high abundances at fertilized sites (Muller 1998). Our findings suggest that the fertilized areas attracted higher numbers of grasshoppers immediately after hatching, demonstrating that grasshoppers can select areas with higher nitrogen even as young hatchlings and stay in these areas through time.

Spatial and temporal variability of grazing in marshes is well documented (Alberti et al. 2007, 2010); however, the extent of these metrics measured for marsh grasshopper grazing on black needlerush is limited, with only few studies addressing this scenario (Carrier 2013; Hunter et al. 2014). We observed some temporal and spatial differences with findings of more grazing in 2010 than 2011 as well as more grazing in the low elevation sites than high elevation site.

Overall, there was more grazing in the 2010 sampling year than the 2011 sampling year. A possible explanation to a

Table 2 Results of two-way ANOVA for treatment, site, and interaction of treatment×site for all measured response variables

Response variable	Treatment		Site		Treatment×site	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
Leaves grazed (%)	6.143	*0.005*	3.370	*0.046*	0.282	0.888
Scars (m^{-1})	0.790	0.462	0.106	0.899	0.454	0.769
Longest scar	17.651	*<0.001*	77.984	*<0.001*	3.274	*0.022*
Leaf grazing degree	22.221	*<0.001*	10.422	*<0.001*	0.603	0.663
Breakage probability	19.278	*<0.001*	22.939	*<0.001*	3.208	*0.024*
Leaf length	2.538	0.094	1.958	0.156	0.584	0.677
Biomass	0.229	0.796	17.238	*<0.001*	1.496	0.224
Living biomass (%)	11.383	*<0.001*	3.469	*0.042*	0.087	0.986
Hopper CPUE	0.514	0.622	0.214	0.660	0.097	0.909

Italicized *p* values indicate significance (*p*<0.05)

higher percentage of grazed leaves in 2010 than 2011 is the colder air temperatures at the beginning of the 2011 grazing season (≈2 °C colder in May 2011 than 2010, NERRS Centralized Data Management Office) which may affect exothermic insects, such as delayed hatching time, increased mortality, and decreased overall activity (Neven 2000). Another possible explanation to the observed differences in grazing between sampling years could be that different sites were used each year. These sites were very close in proximity; however, there could have been some underlying environmental factors driving uneven distribution of grasshoppers, throughout the marsh, that contributed to our observed annual differences in grazing (Carrier 2013).

Elevation and distance from water bodies in salt marshes is known to drive changes in many environmental variables (Adams 1963; Alberti et al. 2010; Christian et al. 1990; Woerner and Hackney 1997) which may in turn influence insect abundances and grazing intensity. Flooding frequency, salinity tolerance, and nutrient content of leaves are all elevation and distance from water body-dependent factors that could alter insect abundance and grazing intensity (Alberti et al. 2010; Christian et al. 1990). In our study, marsh grasshoppers were more abundant at the lower elevations (i.e., closer to the bayou) than at the higher elevation site further from bayou. These findings are interesting because the location of egg deposition is poorly understood for marsh grasshoppers. It can be suggested from this study that marsh grasshoppers either hatch in lower elevation sites or quickly migrate to these lower elevation sites after hatching; however, further investigation is needed.

Nutrient loading into salt marshes is continually increasing through anthropogenic nutrient loading (Howarth et al. 2002; Nixon 1995). Inevitably, some of these anthropogenically derived nutrients will be assimilated into plant material, and this study shows that this may alter grazing pressure on black needlerush through grasshopper grazing. This information is beneficial for managers who are considering water quality goals for nutrient inputs into marshes and/or using black needlerush marshes as nutrient filters. While the decreases in plant health were minimal with increased fertilization-induced grazing, this experiment was only conducted over one grasshopper-grazing season. Grazing frequency and intensity was higher in fertilized areas of the marsh, and this finding suggests that continual fertilization of the same areas could lead to cumulative increases in grazing pressure over time which may cause declines in black needlerush health.

Conclusion

In conclusion, our results suggest that eutrophication can increase grazing in black needlerush salt marshes. However, the effects of increased grazing on plant health were small. A probable cause to the lack of significant decreases in plant health can be attributed to the morphology of black needlerush leaves and relatively short-term nature of this project. Grasshopper abundance was also higher in fertilized treatments relative to unfertilized. Fertilizer was added for only one grazing season, so the long-term effects of increased grazing as a result of fertilization cannot be determined from this study. Our research does show that grazing is increased with fertilization, thereby strengthening the need for longerterm fertilization experiments paired with grazing and plant health measurements to further our knowledge of the effects of anthropogenic eutrophication on salt marshes. Managers can use the information from this project to better understand the potential effects of eutrophication on their respective marsh conservation and restoration projects.

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