Timing of disturbance affects biomass and flowering of a saltmarsh plant and attack by stem-boring herbivores

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Abstract. In salt marshes, disturbance by wrack (floating mats of dead vegetation) is common and affects plant productivity and species composition, but little is known about how the timing of disturbance mediates these effects, nor how it interacts with herbivory. Using a field experiment on the Georgia coast, we simulated the effects of wrack disturbance at different times of the year on the marsh grass Spartina alterniflora and its stem-boring herbivorous insects. The timing of disturbance throughout the growing season strongly affected fall biomass, stem height, the proportion of stems flowering, and the proportion of stems colonized by stem-boring herbivorous insects. End-of-season biomass in plots disturbed in March did not differ from undisturbed controls, but biomass was reduced by 50% in plots disturbed in May, and by over 90% in plots disturbed in September. Disturbance in March and May stimulated flowering, but disturbance later in the growing season suppressed it. Plots disturbed late in the growing season had a low frequency of stem-boring herbivores. Stems containing stem borers rarely flowered. These results indicate that the timing of disturbance matters in coastal salt marshes. Late-season disturbances had the strongest effects on S. alterniflora and its herbivores. Disturbances early in the growing season did not affect end-of-season biomass, and stimulated flowering, suggesting parallels between fire disturbance in grasslands and wrack disturbance in salt marshes. Late-season disturbance did reduce herbivory by stem-boring insects, but not enough to compensate for the direct effects of disturbance on the plants. Future studies of disturbance in salt marshes should consider how the timing of experimental disturbance treatments relates to the timing of natural disturbances.

Key words: disturbance; flowering; phenology; plant population and community dynamics; plant–herbivore interactions; primary production; stem borer; wrack.

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INTRODUCTION

Natural disturbances play important roles in many ecosystems (Pickett and White 1985), and the outcome of disturbances is often affected by their timing (Crawley 2004). In some cases, as with fire, the intensity of the disturbance may vary seasonally (Whelan 1995). In other cases, the timing of the disturbance affects which life-history stages of species in the community are affected (Fill et al. 2012), and the time remaining in the growing season for species to recover (Shepherd et al. 2012). In terrestrial plant communities, the effects of fire and drought on growth and flowering often vary with the time of year in which the disturbances occur (Waldrop et al. 1992, Craine et al. 2012, Shepherd et al. 2012). Similarly, hydrological disturbances to aquatic communities have different effects at different times (Peterson et al. 1990, Peterson and Stevenson 1992).

In coastal habitats, one of the most common natural disturbances is from wrack, dead plant material that is deposited in the intertidal by
waves and tides (Pennings and Bertness 2001, Orr et al. 2005, Li and Pennings 2016). Wrack patches deposited low in the intertidal may be moved daily by high tides; patches deposited high in the intertidal or into the supra-tidal may remain in place indefinitely, decomposing over a period of months. In salt marshes, wrack disturbance may contribute to creekbank erosion (Lottig and Fox 2007), and can kill plants and alter plant community composition (Bertness and Ellison 1987). Wrack can also benefit plants by increasing soil nutrients and reducing porewater salinities (Pennings and Richards 1998). Wrack is generated most profusely as plants senesce in the fall and winter, and so disturbances are most common in the spring; however, wrack patches are periodically moved about by the tides, and so new disturbances can occur at any time during the growing season (Bertness and Ellison 1987, Valiela and Rietsma 1995). Whether the timing of disturbance matters has not been studied.

Disturbance also affects herbivores (Denno et al. 1980). Depending on whether herbivores are more or less affected by disturbance than plants, disturbance can interact with herbivory in different ways (Menge and Sutherland 1987, Hunter and Price 1992). Salt marshes are prone to drought-induced die-back of vegetation, and drought stress appears to increase the vulnerability of some plants to herbivory by snails and stem-boring insects (Moon and Stiling 2002, Goranson et al. 2004, Silliman et al. 2005, Gaeta and Kornis 2011), but effects of wrack on herbivory have not been examined. Moderate wrack disturbance is unlikely to affect snails, but stem-boring insects that have limited mobility are likely to die if the plant stems that they inhabit are killed by wrack. Moreover, whether new stems that grow following a disturbance are colonized by stem-boring insects will depend on oviposition preferences of female insects and the time of year at which eggs are laid.

We conducted a field experiment in creekbank habitat in coastal Georgia to explore the importance of the timing of wrack disturbance on end-of-season plant biomass, plant flowering, and the prevalence of stem-boring herbivores. We hypothesized that plants would be least affected by disturbances occurring early in the growing season because the plants would not yet be flowering and would have more time to regrow than if they experienced disturbances late in the growing season. We expected stem-boring herbivores to also be affected by disturbance, but we knew too little about their life cycles or preferences for larger (older) vs. smaller (younger) stems to predict in what way.

**Materials and Methods**

We worked in a salt marsh along Dean Creek at the southern end of Sapelo Island in Georgia, United States (31.39° N, 81.28° W). The dominant plant in low and middle marsh elevations was the C₄ grass *Spartina alterniflora*. In Georgia, this grass grows year-round, but above-ground biomass is highest between June and November (Gallagher et al. 1980). Flowering peaks around October (S. C. Pennings, personal observation). During the growing season of the study year (2015), there were no dramatic changes in sea level, droughts, or extremely cold days (Appendix S1: Tables S1–S2, Fig. S1), and average conditions were not unusual compared to the previous decades (Więski and Pennings 2014).

*Spartina alterniflora* is attacked by several stem-boring insects (Pfeiffer and Wiegert 1981, Stiling and Strong 1983), but because of their cryptic lifestyle they are often overlooked in ecological studies. We focused on stem-boring herbivores in this manuscript because their presence and abundance over an extended period can be easily quantified by a one-time collection, and because their intimate associate with plant stems makes them likely to be affected by disturbances that harm plants (Armitage et al. 2013).

The Georgia Coastal Ecosystems (GCE) Long-Term Ecological Research Program has documented wrack disturbance to eight sites in coastal Georgia dominated by *S. alterniflora* since 2001 (Li and Pennings 2016). We conducted a field experiment at one of these sites, GCE 6 (Appendix S1: Fig. S2), in 2015. We established 72 plots, each 1 × 1 m, in *S. alterniflora* along Dean Creek at GCE 6. We simulated wrack disturbance on four different dates by covering selected plots with 10 cm of wrack collected nearby on 18 March, 27 May, 15 July, and 7 September 2015. The wrack bent over tall plant stems, typically breaking them near the base. Because the plots were smaller than natural wrack patches, the wrack was fixed to the marsh
surface with string so that it would not wash away. We also simulated the mechanical disturbance created by wrack, but without the input of allogenic organic matter (Pennings and Richards 1998) by clipping selected plots 1 cm above the soil surface on the same dates. This created a total of nine treatments (4 dates × 2 disturbances plus a control) with eight replicates each. We measured plant heights in a 50 × 50 cm quadrat centered in each plot before the first treatments were conducted in March, again on 9 May, and every 2 weeks thereafter until plots were harvested in October. We also noted whether each stem was flowering, and used this information to estimate stem biomass, using an allometric regression equation (Wiśniski and Pennings 2014) for the creekbank zone of GCE site 6, which is immediately adjacent to our experimental site:

\[ \text{Ln (Biomass (g))} = -6.095 + 1.760 \times \text{Ln (height (cm))} + 0.212 \times (\text{flowering} - 0) \] 

For creekbank *S. alterniflora*, this allometric relationship correlates strongly \((R^2 = 0.97)\) with plot-level biomass (S. C. Pennings, unpublished data).

The center 50 × 50 cm of each plot was harvested by clipping stems at the soil surface on 5–6 October 2015. We measured the height of each stem, noting whether it was flowering, and counted the number of stem-boring insects present in each stem by splitting the stem open. In some cases, an emergence hole and hollow interior showed that a stem borer had been present but had already emerged, in which case the stem was scored as “bored,” but the number of stem borers was not assigned. Representative larvae were reared to adulthood to identify stem borer taxa. Stems were dried for 3 d at 60°C and weighed. Biomass in the different treatments was compared using one-way analysis of variance (ANOVA), with post hoc Tukey’s HSD (honest significant difference) tests to compare individual treatments. Percent data (% of stems bored or flowering) were converted to proportions, arcsine-square-root-transformed, and compared with one-way ANOVA, with post hoc Tukey’s HSD tests to compare individual treatments. Experimental data are available online (Li 2016).

To determine how stem borers affected plant end-of-season biomass, at the beginning of October we collected 50 *S. alterniflora* shoots with stem borers and another 50 without from the area surrounding the experimental plots. Whether or not shoots have stem borers can usually be determined by examining the growing tip of the plant (Appendix S1: Fig. S3). We measured the height and recorded the flowering status of each shoot, split it open, and counted the number of stem-boring larvae present. Shoots were dried for 3 d at 60°C and weighed. Because most of the stems with stem borers were not flowering, we compared the effect of stem borers on the height–mass relationship of non-flowering stems \((n = 40\) with stem borers and 40 without) using analysis of covariance (ANCOVA) with stem borers as the treatment and height as the covariate.

**Results**

Wrack and clipping treatments reduced estimated aboveground biomass to zero on each treatment date (Fig. 1A). Otherwise, estimated biomass increased steadily with time, except for a slight decline in August, which may have been due to stem borers or to high densities of grasshoppers that inflicted obvious damage to the plants at that time. (Both herbivores can affect measurements of plant height: stem borers by killing the meristem and grasshoppers by consuming parts of the upper leaves.) Plots disturbed in March converged with control plots by October, but plots disturbed later did not fully recover in biomass by October.

October standing biomass differed among treatments (Fig. 1B). Plots disturbed in March did not differ from undisturbed controls. Plots disturbed in May had only 50% of the biomass of control plots, and plots disturbed in July and September had even less biomass.

The percent of stems attacked by stem-boring insects depended on when plots were disturbed by wrack (Fig. 2A). Plots disturbed in March had the same frequency of stem borers as control plots, but plots disturbed in July and September had very low stem borer attack rates (Fig. 2A). Stems shorter than 40 cm rarely housed stem-boring insects (Appendix S1: Fig. S6).

The percent of stems that flowered also depended on when plots were disturbed by wrack (Fig. 2B). In control plots, about 5% of stems flowered. In contrast, in plots disturbed in March and May, up to 20% of the stems flowered, although the pairwise comparisons with the control plots...
were not always statistically significant. In plots disturbed in July and September, none of the stems flowered. Stems shorter than 60 cm rarely flowered (Appendix S1: Fig. S7).

Disturbance altered the height–frequency distribution of the stems in the plots (Appendix S1: Fig. S4). Plots disturbed in March and May had height–frequency distributions qualitatively similar to control plots with the most frequent stem heights in the 60–90 cm size range. Plots disturbed in July had a modal height of 20–60 cm, and plots disturbed in September had a modal height of 10–40 cm. Control plots lacked any stems shorter than 30 cm, but all the disturbed plots had some stems in this size range.

Stems containing stem borers rarely flowered (1 out of 50 vs. 10 out of 50 stems lacking stem borers) and were heavier at a given height than stems lacking stem-boring insects (Fig. 3). Almost half (42%) of the bored stems no longer contained a stem-boring insect in October, but the history of stem boring was revealed by a hollow stem interior and an emergence hole. The remaining bored stems contained stem borers, mostly fly larvae (38%, usually in the upper part of the stem) or moth larvae (22%, usually in the...
A few stems (6%) contained another stem-boring species that we did not identify (likely *Calamomyia alterniflora* [Diptera: Cecidomyiidae]). Most of the stems had only one larva inside, but we found one stem with a fly larva and a moth exuvia, and another with 16 small moth larvae.

We identified the two most common stem borer species by raising representative individuals to adulthood (Appendix S1: Fig. S5). The fly, *Chaetopsis apicalis*, can be distinguished by the single dark band at the apex of the wing (Appendix S1: Fig. S5C). The moth was in the family Pyralidae (Appendix S1: Fig. S5F), which contains a number of other stem-boring species. The moth larvae were approximately two to four times longer than the fly larvae, and consumed much more of the stem core than did the fly larvae.

**DISCUSSION**

In our experiment, the timing of disturbance affected how *Spartina alterniflora* and its stem-boring insects responded. Wrack disturbance reduced fall biomass and stem height, altered the proportion of stems flowering in the fall, and reduced the frequency of attack by stem borers, but these effects strongly depended on the timing of disturbance.

Wrack disturbance significantly and strongly reduced plant biomass and modal stem height. The effect of wrack disturbance on fall standing biomass depended on the timing of the disturbance. Plants recovered from wrack disturbance that occurred in March, with biomass and stem heights comparable to the control treatment by the time we harvested the experiment in October. In contrast, plants did not fully recover from disturbance that occurred later in the growing season. In nature, wrack is produced by the death of *S. alterniflora* stems, which happens primarily over the winter, and so wrack abundance is greatest earlier in the growing season and declines into the fall (Bertness and Ellison 1987, Valiela and Rietsma 1995). As a result, disturbances are likely to be more common earlier in the growing season. Patches of wrack are moved about by high tides; however, especially if they do not lodge high in the intertidal, so new disturbances can happen at any time of the year.

Recovery of vegetation from wrack disturbance is a function of several variables. The thickness of the wrack mat determines whether or not plants can grow through it or cannot recover until the wrack either washes away or decomposes (Bertness and Ellison 1987, Valiela and Rietsma 1995, Brewer et al. 1998). How long the wrack remains in place determines whether some plants survive the disturbance or all are killed (Bertness and Ellison 1987, Valiela and Rietsma 1995). The size of the wrack patch and the level of abiotic stress in the patch determines how long it takes plants to fill a disturbance-generated unvegetated area by growing in from the sides (Shumway and Bertness 1994, Brewer and Bertness 1996, Angelini and Silliman 2012). The timing of the disturbance relative to plant phenology (Gallagher et al. 1980) affects the severity of the disturbance, because plants translocate resources from belowground rhizomes to aboveground shoots at the start of the growing season. Thus, a disturbance in July removes a greater percentage of the plant’s resources than a disturbance in March. Finally, the timing of the disturbance also affects how much time remains in the growing season during which plants can recover (Alber et al. 2013). In our experiment, wrack patches were relatively small...
and thin, and we observed that vegetation was able to recover by growing through the wrack. Although our experimental manipulation mimicked the most common type of wrack disturbance at our study site, we also observed larger, thicker mats of wrack that completely killed vegetation and produced unvegetated areas in the S. alterniflora zone that appeared to take a few years to recover. Exploring the interactive effects of the timing and size of wrack disturbance was outside the scope of this project, which focused on the timing of relatively modest wrack disturbances, but deserves attention in the future.

Wrack represents not only a disturbance but also a source of nutrients and protection from physical stress (Pennings and Richards 1998). These positive effects of wrack may be more important in the more-stressful high marsh than in the lower marsh elevations dominated by S. alterniflora (Pennings and Richards 1998). We found no major differences between the wrack and clip treatments, indicating that the primary way that wrack affected S. alterniflora biomass and height in this experiment was by killing stems, rather than by providing a nutrient subsidy or ameliorating physical stress. There were trends suggesting that the May wrack and clip treatments had different effects on stem borers and flowering (Fig. 2), but these trends were not statistically significant.

Wrack disturbance early in the growing season appeared to stimulate flowering, although few of the individual means comparisons with the control treatment were statistically significant due to moderate replication and high variability. Wrack and clipped treatments both responded similarly, indicating that the increased flowering was a response to disturbance rather than a nutrient subsidy. Other studies have also found that disturbance and stress can increase flowering in S. alterniflora (Shumway and Bertness 1994, Daleo et al. 2015, Liu et al. 2016). In terrestrial grasslands, fire at the right phenological stage often stimulates flowering (Fill et al. 2012, Shepherd et al. 2012). Flowering in response to disturbance is likely to be adaptive if the disturbance frees up resources and increases performance of seedlings, but plants may only be able to increase effort to sexual reproduction if the disturbance occurs at the right phenological stages. Alternatively, disturbance or stress can also be an indicator of a

suboptimal habitat that causes organisms to shift resources away from vegetative growth into sexual reproduction in order to disperse (Hamilton et al. 1987, van Kleunen et al. 2002, Crosby et al. 2015). Disturbances that occurred later in the growing season led to almost no flowering, likely because shoots simply did not grow large enough to flower (Appendix S1: Fig. S7).

Consumers play important roles in controlling the production of plants in coastal ecosystems (Silliman and Bortolus 2003). We expected that stems with stem borers would have less biomass than un-bored stems of the same height, due to consumption of plant material by the stem borer, but the data indicated that they were instead heavier. The most likely explanation for this result is that stem borers killed the upper, thinner parts of the stem, leaving only the thicker basal region to be measured. An alternative possibility is that the stem borers preferentially selected stems with larger diameters that might provide more food or better protection from parasitoids. How stem borers affect S. alterniflora production at the stand level remains unexplored; however, the lack of stem borers in plots disturbed in July and September did not allow plants to compensate for wrack disturbance.

Consistent with past reports, we observed that S. alterniflora stems containing stem borers usually did not flower (Grevstad et al. 2004). In cases where flowering did occur, it is likely that the stem borer had colonized the stem too recently to disrupt the development of the flower. As a result, we hypothesize that widespread attack by stem borers might significantly reduce sexual reproduction in S. alterniflora. Because stem borers also limit the growth of the stem, they may contribute to loss of vegetation in areas where S. alterniflora is already stressed by other factors (Gaeta and Komis 2011). In general, because stem borers are difficult to directly observe, they have been overlooked in studies of salt marsh plant ecology (but see Stiling and Strong 1983), and their role in mediating plant reproduction and vegetative growth deserves further attention.

Natural disturbances can interact with the mobility or phenology of consumers to affect the abundance of consumers (Armitage et al. 2013). By killing plant stems, wrack disturbance killed any stem borers present at the time of the disturbance. Everything else being equal, older
stems will have had more time to accumulate stem-boring larvae than will the younger shoots that emerged from the disturbed plots. This seems unlikely to explain why plants disturbed in May had low levels of stem borers in October, 4 months later. More likely, the re-growing stems were too short (Appendix S1: Fig. S6) to be attractive to egg-laying females during the time that females were laying eggs. Armitage et al. (2013) also found that clipping of aboveground *S. alterniflora* biomass in a Texas marsh early in the growing season (April) reduced the abundance of larval stem borers. Similarly, in agricultural and forest systems, burning disturbance can kill 95% of the stem borers (Adesiyun and Ajayi 1980), and disturbance by snow may also promote the mortality of some stem borers (Ayres and Lombardero 2000).

A major gap in our understanding of how stem borers affect *S. alterniflora* is that we do not know how stem borers affect clonal integration among shoots (Pennings and Callaway 2000). Herbivory by stem borers (Utsumi and Ohgushi 2008, Forrioni 2011, Stephens and Westoby 2015) or by chewing herbivores (Cain et al. 1991, Piqueras 1999) may weaken other shoots on the same clone if the attacked stem becomes a resource sink; alternatively, plants may be able to withdraw resources from attacked stems and divert them to support growth and flowering of other shoots on the same clone. As a consequence, we cannot extrapolate from the performance of individual bored stems to estimate the net effect of stem-boring insects on the clone as a whole.

Stem borers in forest and agricultural systems have been well studied (Adesiyun and Ajayi 1980, Ayres and Lombardero 2000, Stephens and Westoby 2015), but fewer studies have focused on stem borers in grasslands. We found that attack by stem borers interacts with disturbance, such that the timing of disturbance affects the probability of attack by stem borers, and that both disturbance and stem borers affect end-of-season standing biomass. Because end-of-season standing biomass is often used to estimate annual net primary productivity in grasslands, our estimates of ANPP could be improved by a better understanding of both disturbance and herbivory.

Previous experimental studies of wrack disturbance in salt marshes have typically applied the wrack at a single date that sometimes did and sometimes did not correspond to the peak time of natural wrack disturbance (Bertness and Ellison 1987, Brewer et al. 1998, Pennings and Richards 1998). Future studies should consider the natural seasonal pattern of wrack disturbance in designing their studies, either initiating treatments when natural disturbance peaks, or incorporating multiple disturbance treatments to represent the range of times when disturbance can occur.

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**LITERATURE CITED**


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