Can The Marsh Migrate? Factors Influencing The Growth Of Spartina Patens In Upland Soil

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CAN THE MARSH MIGRATE?
FACTORS INFLUENCING THE GROWTH OF *SPARTINA PATENS*
IN UPLAND SOIL

BY

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B.A. Bard College, 2010

THESIS

Submitted to the University of New England
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This thesis project is dedicated, with love, to my grandparents,
*Vincent and Evelyn Dowling.*
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ABSTRACT

CAN THE MARSH MIGRATE?
FACTORS INFLUENCING THE GROWTH OF *SPARTINA PATENS*
IN UPLAND SOIL

by

Tessa M. Dowling

University of New England, August, 2018

Although high elevation salt marsh plants, such as *Spartina patens* (salt hay) can cope with accelerated sea level rise by migrating inland, it is not well known whether environmental factors, such as soil, plant litter, and salinity, will influence the ability of *S. patens* to colonize upland forest areas. For one growing season, I tested how *S. patens* vegetative growth (the final number of stems, aboveground stem biomass, and belowground rhizome biomass) and reproduction (presence of flowers) responded to upland or marsh soil, the presence or absence of plant litter, and 4.5ppt or 14.5ppt salinity levels. In order to determine if the source location of the plant influenced their response to treatment effects, I collected *S. patens* plants from three Maine salt marshes in the townships of Scarborough, Biddeford, and Wells. Litter and salinity treatments did not significantly affect vegetative growth, and they only affected flowering in a three-way interaction with site. All vegetative and reproductive measures were significantly affected by soil and the site x soil interaction - *S. patens* collected from Biddeford and Wells grew significantly less in the upland soil compared to the marsh soil, but Scarborough plants grew equally well in both soil treatments. One possible explanation for why plants from the three sites responded differently to soil
treatments was that the Scarborough site had a significantly lower percent soil organic matter content, and therefore, was more similar to upland in soil organic matter content than the other two sites. These results suggest that *S. patens* populations from a site with low soil organic content will be more successful adjusting to upland soil than plants from high soil organic matter sites, which would give those populations accustomed to low organic matter an advantage when migrating inland. The ability to identify *S. patens* sites that will successfully migrate inland, by measuring soil organic content or other site characteristics, is vital if conservation efforts are going to protect the *S. patens* populations most likely to persist in the face of sea level rise.
INTRODUCTION

Accelerated sea level rise has led to increased rates of salt marsh loss world-wide (Warren and Niering 1993; Stammermann and Piasecki 2012; Fagherazzi 2013). Loss of salt marshes can have negative environmental effects, such as decreasing waterfowl nesting and feeding habitat (Clausen and Clausen 2014), and cause negative effects on coastal human infrastructure due to the increased impact of storm surges and the decrease in shoreline stability (Shepard et al. 2011). Sea level rise also causes detrimental shifts in salt marsh vegetative communities (Short et al. 2016). Typical New England salt marsh communities are divided into two zones; a high marsh zone dominated by the perennial grass salt hay (*Spartina patens*), and a low marsh zone dominated by smooth cordgrass (*Spartina alterniflora*) (Pennings and Bertness 2001; Lonard et al. 2010). An intolerance to high salinity and inundation levels prevents *S. patens* from colonizing the low marsh, and interspecies competition generally prevents *S. alterniflora* from spreading into areas dominated by *S. patens* (Bertness and Ellison 1987; Ungar 1998). However, sea level rise has an indirect negative impact on *S. patens* by facilitating the movement of *S. alterniflora* populations into the high marsh *S. patens* zone (Donnelly and Bertness 2001; Watson et al. 2016). With increasing rates of sea level rise, *S. patens* is squeezed between the encroaching *S. alterniflora* and the adjacent upland (Tono and Chmura 2013), unless it can migrate inland.

Evidence of trees stumps within salt marshes and analysis of historic photos illustrate that salt marshes have migrated inland in the past (Kirwan et al. 2016; Raabe
and Stumpf 2016). However, what is not clearly understood is whether environmental factors, including soil composition, the presence of plant litter, and salinity, have the potential to impede *S. patens* inland migration. The upland that migrating *S. patens* encounters will not match its native marsh environment, in part because upland soil is unlikely to convert into marsh soil as quickly as vegetation can move inland (Anisfeld et al. 2017). For example, upland soil organic matter content can take longer than three years to match the organic content of natural salt marsh soils under created salt marsh conditions (Moy and Levin 1991). Soil organic matter is particularly important to marsh plant growth because it pools nutrients and promotes nutrient fixation (Langis et al. 1991; Callaway 2000), but it takes time for organic matter to build-up in soils changing from an upland into a marsh environment (Moy and Levin 1991), which could deter *S. patens* growth.

Research suggests that plant litter and salinity can also decrease vegetative growth. The presence of salt marsh litter, such as wrack, can physically block stem growth leading to declines in *S. patens* aboveground biomass (Tolley and Christian 1999). Forest plant litter, that *S. patens* might encounter when migrating into the upland, also can inhibit plant growth by acting as a physical barrier to stem emergence and by blocking sunlight (Xiong and Nilsson 1999; Sayer 2006). Salinity levels can cause salt stress to *S. patens* (Pezeshki and DeLaune 1993) and levels above 7ppt can limit aboveground and belowground growth (Ewing et al. 1995; Snedden et al. 2015).

Few published studies have investigated how plant litter and salinity affect salt marsh plant reproduction. However, Li and Pennings (2017) found that the timing of
wreck litter disturbance influenced the effect of litter on *S. alterniflora* reproduction; wreck litter placed over *S. alterniflora* stems early in the growing season stimulated flower production, while wreck litter placed over stems later in the growing season decreased the number of stems that produced flowers. When Xiao et al. (2011) studied *S. alterniflora* reproduction, they found that, within one growing season, a salinity level of 30ppt caused flower biomass to decrease compared to a salinity level of 15ppt, but that the number of stems that produced flowers did not change between the two salinity treatments. Since *S. patens* is a salt marsh plant in the same genus as *S. alterniflora*, it is possible that *S. patens* would have a similar response to plant litter and salinity treatments as *S. alterniflora*, and show a decline in flowering when litter is present late in the growing season, but not have the number of stems with flowers decrease due to salinity levels.

As a species, *S. patens* can grow in a wide range of habitats and has a large geographic range (Broome et al. 1995; Bush and Houck 2008). Within that range, *S. patens* shows high intraspecies variation in salt tolerance (Hester et al. 1996; Pezeshki and DeLaune 1997), waterlogging (Burdick and Mendelssohn 1987), and biomass allocation (Brewer and Bertness 1996). The growth response of *S. patens* to soil, plant litter, and salinity also might vary among populations from different salt marsh sites, and, therefore, site is another important factor which could determine how plant growth will respond to the need to migrate inland.

To test the influence of environmental factors on the migration potential of *S. patens* into the upland, I used a manipulative experiment to study the effects of four
factors - soil, plant litter, salinity, and site- on *S. patens* vegetative growth and reproduction. I designed the experiment with two soil treatments, two litter treatments, two salinity treatments, and three site treatments. I predicted that 1) the upland soil treatment would decrease *S. patens* vegetative growth compared to the marsh soil treatment, 2) the plant litter would decrease *S. patens* vegetative growth and reproduction because the litter would be present throughout the growing season, 3) the higher salinity level would cause a decrease in vegetative growth compared to the low salinity, and 4) *S. patens* from the three sites would respond differently to the other experimental factors (soil, litter, and salinity) due to different tolerance levels among the *S. patens* populations. Results from my experiment will provide insight into whether environmental factors will limit the migration of *S. patens* into the upland. Studying the details of marsh migration is important if we want to design successful management tools to assist marsh migration and help salt marshes persist into the future despite sea level rise.
METHODS

Study Species Collection

The *S. patens* used in this study was collected in June 2017 from three southern Maine salt marshes located in the townships of Scarborough (43°33'52.6"N 70°22'25.9"W), Biddeford (43°27'23.1"N 70°22'52.0"W), and Wells (43°19'58.5"N 70°32'45.0"W) (Fig. 1). The Scarborough site was located approximately 11km north of the Biddeford site and approximately 30km north of the Wells site. *S. patens* was extracted with spades from the marsh sites in approximately 8.5cm diameter by 21cm deep plugs, containing approximately 30 stems, and including the intact root mass and soil. Eighty *S. patens* plugs were collected from each marsh site (240 total), placed in plastic bins with saltwater, and then transported back to the University of New England (adjacent to the Biddeford collection site).

Study Design

I divided the 80 plugs from each of the three sites among the three other treatment factors such that there were ten replicates for each treatment combination (3 site x 2 soil x 2 litter x 2 salinity x 10 = 240). The two soil treatments were marsh and upland, and were created by planting *S. patens* plugs in fabric pots (having a volume of 19L, a diameter of 30cm, and a depth of 25cm) filled with either marsh soil from the creeks at each of the three marsh sites, or with upland soil from the top 30cm of a forest, dominated by *Quercus rubra* and *Acer rubrum*, in Scarborough, ME (43°37'51.0"N 70°24'02.2"W, Fig. 1). The two plant litter treatments were the presence or absence of
litter. For the 120 plugs in the litter present treatment, the plant litter was placed over the surface of the soil around the plug and the composition of the litter corresponded to the soil treatment, such that the litter placed over marsh soil was clipped dead *S. patens* from the three marsh sites and the litter placed over the upland soil was *Quercus* and *Acer* leaves from the upland forest site. For the 120 plugs in the litter absent treatment the soil was left exposed. To create the salinity treatments, I assigned 24 pots containing *S. patens* plugs to one of ten 1.8m diameter by 0.4m deep plastic wading pools, such that each pool contained two replicates of each site x soil x litter treatment combination. Half the wading pools had a low salinity treatment, created by adding 500g of aquarium salt to approximately 70L of freshwater, and half of the wading pools had a high salinity, created by adding 3120g of aquarium salt to approximately 70L of freshwater. The low salinity treatment averaged 4.5ppt ± 0.2 (standard error of the mean) over the course of the growing season and the high salinity averaged 14.5ppt ± 0.5. Water levels within the pools were maintained at a depth of at least 5cm, with occasional variations due to rainfall, throughout the study, which ran from June until September 2017.

I used four variables to quantify *S. patens* response to treatment factors: the final number of stems, aboveground stem biomass, and belowground rhizome biomass were used as indicators of vegetative response, and flowering was used to measure reproduction. The final number of stems corresponded to the number of stems in each pot at the end of the experiment. After counting, I collected the aboveground biomass by clipping the stems where they exited from the soil surface, dried the stems to a constant mass in a 60°C oven, and then recorded aboveground biomass in dried grams per pot.
(g/pot). For belowground biomass, I collected only the rhizomes that grew during the experiment by clipping the rhizomes where they exited from the *S. patens* plug (which had maintained its structural integrity throughout the study). I dried the rhizomes to a constant mass in a 60°C oven, and then recorded each rhizome biomass in dried g/pot. Flowering occurred in August 2017, and I recorded the number of flowers per *S. patens* stem, the number of stems with flowers per pot, and whether flowers were present in each pot within each treatment combination.

To understand whether differences in soil properties among sites could explain site response differences to upland soil, I collected 12 soil samples (measuring approximately 8.5cm in diameter and 21cm deep) from each of the three *S. patens* collection sites. The samples were collected in April 2018 and were sent to the Analytical lab and Maine Soil Testing Service, located at the University of Maine-Orono campus, and tested for organic matter content through loss of ignition at 375°C for 2 hours (Schulte and Hoskins 2011).

**Data Analysis**

I analyzed the main and interactive effects of the four environmental factors on plant response using R statistical software (Version 3.3.1 2016). All response variables were analyzed separately, and different modeling approaches were used for the three continuous vegetative growth variables (final number of stems, aboveground stem biomass, and belowground rhizome biomass) than for the categorical reproductive variable (presence or absence of flowers in a pot), because of non-normality issues with continuous reproductive measures, such as the number of flowers per pot. Stem biomass
and rhizome biomass were logarithmically transformed to meet model assumptions of normality. I accounted for variations among *S. patens* plugs that could confound the treatment effects by assessing several potential covariates using multiple linear regression. Three potential covariates were assessed for both the final number of stems and aboveground stem biomass: the number of initial stems per pot, the number of days the stems grew per pot prior to taking measurements, and the presence or absence of flowers (Appendix A I-II). Three covariates were assessed for belowground rhizome biomass: the number of initial stems per pot, the number of days the rhizomes grew per pot, and the depth the soil around the plug subsided during the experiment, exposing some of the rhizomes (Appendix A III). I used the residuals from the covariate regression models (retaining significant covariates, significant covariate interactions, and any non-significant term associated with significant higher-order interactions) as the adjusted response values in the ANOVAs (3 site x 2 soil x 2 litter). These crossed factors were nested within pools, which, in turn, were nested within the two salinity treatments. If treatment effects were significant (*p* ≤ 0.05), then I conducted multiple F-test (ANOVA) pairwise comparisons to analyze differences within the effect using sequential Bonferroni-corrected alpha levels (e.g. starting at *α* = 0.05/9 ≈ 0.01 for soil x site interactions). Poisson log-linear modeling was used to test the significance of treatment effects on the presence or absence of flowering (*p* ≤ 0.05), and multiple comparisons were conducted using odds-ratio tests. I compared soil organic matter among sites with an ANOVA (*p* ≤ 0.05), and then tested for significance between pairs of sites using a
series of F-tests (ANOVAs) and sequential Bonferroni corrected alpha levels (starting at $\alpha = 0.05/3 \approx 0.02$).
RESULTS

The final number of stems, which averaged 104 ± 2.4 (standard error of the mean) stems at the end of the growing season, was generally lower in the upland soil compared to the marsh soil, although the trend was not consistent across all sites. The final number of stems (adjusted for the number of initial stems, the number of grow days, and the presence of flowering) significantly differed by soil type and site x soil interaction (Table 1, Fig. 2). Specifically, the adjusted number of stems was significantly lower in upland soil than in marsh soil for plants from the Biddeford (ANOVA $F_{1,78} = 8.07, p < 0.01$) and Wells (ANOVA $F_{1,78} = 40.91, p < 0.01$) sites, but not for plants from Scarborough (ANOVA $F_{1,78} = 2.86, p = 0.10$). When comparing sites within the upland soil treatment using sequential Bonferroni adjusted alpha levels (Fig. 2), Scarborough had a significantly higher count than Wells in the upland soil (ANOVA $F_{1,78} = 22.13, p < 0.01$), but Biddeford did not differ from the other two sites ($F_{1,78} = 4.36, p = 0.04$; ANOVA $F_{1,78} = 6.75, p = 0.01$).

Significant treatment effects were the same for aboveground stem biomass (adjusted for the number of initial stems, the number of grow days, and the presence of flowering) and belowground rhizome biomass (adjusted for the number of initial stems, the number of grow days, and the depth of soil subsidence) as they were for final the number of stems: there was a significant soil treatment effect and site x soil interaction (Tables 2 and 3). Aboveground and belowground biomass trends were also consistent with the final number of stems findings, with lower growth overall in the upland soil as
compared to marsh soil (Fig. 3 and 4), except for plants from Scarborough (ANOVA $F_{1,78} = 9.71, p < 0.01$ aboveground and $F_{1,78} = 13.09, p < 0.01$ belowground for Biddeford; ANOVA $F_{1,78} = 13.17, p < 0.01$ aboveground and $F_{1,78} = 15.17, p < 0.01$ belowground for Wells; ANOVA $F_{1,78} = 3.25, p = 0.08$ aboveground and $F_{1,78} = 0.10, p = 0.76$ belowground for Scarborough). In addition, plants from Wells had lower biomass than Scarborough within the upland soil treatment (Fig. 3, ANOVA $F_{1,78} = 8.42, p < 0.01$ for aboveground biomass; Fig. 4, ANOVA $F_{1,78} = 9.78, p < 0.01$ for belowground biomass). There were no significant differences between Biddeford and the other two sites within the upland soil treatment, although there was a greater decrease between the adjusted biomass of the Biddeford plants compared to the Wells plants than between the Biddeford plants and the Scarborough plants (Fig. 3, ANOVA $F_{1,78} = 1.18, p = 0.28$ and $F_{1,78} = 3.04, p = 0.09$ for aboveground biomass; Fig. 4, ANOVA $F_{1,78} = 0.95, p = 0.33$ and $F_{1,78} = 3.86, p = 0.05$ for belowground biomass). The aboveground stem biomass averaged $7.6 \pm 0.2$ g/pot and ranged from 1.5 to 27.5 g/pot. The average belowground rhizome biomass was approximately a quarter of the average stem biomass and ranged from 0.02 to 7.9 g/pot.

Reproduction (as reflected in the number of pots per treatment combination that had flowers), similar to the vegetative growth variables, decreased overall in the upland soil treatment but to varying degrees among sites. Flowers grew in 62 of the 240 pots (approximately 25% of pots). The number of stems with flowers per pot ranged from 0 to 7 and the total number of flowers per pot, since some stems grew more than one flower, ranged from 0 to 15. Reproduction was significantly influenced by site, soil, the
site-soil interaction, and the three-way interaction of site, litter, and salinity (Table 4).
The interaction between site and soil reflected what was found for the vegetative growth
variables: reproduction by plants from Biddeford and Wells was lower in upland soil than
in marsh soil, while flowering for Scarborough plants was similar between the two soil
treatments (Fig. 5). However, when I compared the reproduction in upland versus marsh
soil treatment within a site, only Biddeford had a significant decrease in number of pots
with flowers (odds ratio = 14.80, 95% CI = 1.81-121.15 for Biddeford; odds ratio = 1.11,
95% CI = 0.46-2.70 for Scarborough; odds ratio = 2.15, 95% CI = 0.71-6.53 for Wells,
where a 95% CI overlapping 1 would support the null hypothesis of no difference).
When reproduction within the upland soil treatment was compared among sites, more
pots from Scarborough contained flowers than either Biddeford (odds ratio = 26.00, 95%
CI = 3.24-208.81) or Wells (odds ratio = 3.78, 95% CI = 1.29-11.06), but Biddeford and
Wells did not significantly differ (odds ratio = 0.15, 95% CI = 0.02-1.27, encompassing
the null hypothesis). There was no consistent pattern in the interaction between litter and
salinity among sites. For example, when litter was present, a higher number of
Scarborough and Wells pots contained flowers in the high salinity treatment compared to
the low salinity treatment, but Biddeford pots had the exact opposite flowering trend with
more pots containing flowers in the low salinity treatment.

I found a significant difference in percent soil organic matter content among sites
(Table 5). The average percent organic matter from the Scarborough site (16.7 ± 2.0%
s.e.m.) was less than half of the average from Biddeford (41.2 ± 2.6%) or Wells (42.2 ±
1.3%). The observed difference in percent organic matter between Scarborough and the
other two sites was significant (ANOVA $F_{1,22} = 57.14$, $p < 0.01$ for the Scarborough-Biddeford comparison, ANOVA $F_{1,22} = 111.70$, $p < 0.01$ for the Scarborough-Wells comparison), but there was no significant difference in percent organic matter between the Biddeford and Wells sites (ANOVA $F_{1,22} = 0.12$, $p = 0.73$).
DISCUSSION

Inland migration enables salt marsh persistence even in the face of marsh submergence due to accelerated sea level rise (Kirwan et al. 2016; Raabe and Stumpf 2016; Schieder et al. 2018). Obstacles to the successful migration of salt marsh plants, including anthropogenic barriers, the resistance of forests to retreat ahead of the salt marsh, and the steepness of adjacent upland slopes, have been documented (Doyle et al. 2010; Feagin et al. 2010; Smith 2013), but no published studies have addressed the effect of soil on the transition of salt marsh plants from the marsh to the upland. Upland soil differs from marsh soil in bulk density, nutrient retention, and organic matter content (Brinson et al. 1995; Callaway 2000; Truog 2016); all characteristics that could deter marsh plant growth in upland soil (Callaway 2000; Reddy and DeLaune 2008). As predicted, I found that *S. patens* overall performed less well in the upland soil than in the marsh soil, and that the response varied depending on the collection site of the *S. patens* plants. I found that *S. patens* collected from two salt marsh sites (Biddeford and Wells) grew significantly less in upland soil compared to marsh soils; for Biddeford plants this decrease was consistent across all three vegetative growth variables (*final number of stems*, Fig. 2; *aboveground stem biomass*, Fig. 3; *belowground rhizome biomass*, Fig. 4) and reproduction (*flowering*, Fig. 5), and for Wells plants the significant decrease occurred for all variables except flowering. Interestingly, I found that the growth of *S. patens* collected from the other marsh site, Scarborough, did not significantly respond to soil treatment. Furthermore, although I had predicted that litter and salinity would impact plant biomass based on results from prior research (Xiong and Nilsson 1999; Ewing et al.
1995), they were relatively unimportant factors in my study, and only influenced reproduction, as reflected in a significant three-way site x litter x salinity interaction for flowering (Table 4).

The variation in plant response to soil treatment found when comparing *S. patens* from the three collection sites (Fig. 2-5) can partly be explained by differences in soil organic matter. The organic matter percentages from all three collection sites fell within reported values for salt marsh soils which range from less than 5% to greater than 45% (Swarzenski et al. 1991; Callaway 2000; Edwards and Proffitt 2003). Wells (42.2%) and Biddeford (41.2%) were close to the high end of the organic content range, while Scarborough (16.7%) was close to the lower end. The organic content in upland soils is generally lower than in a marsh (Brinson et al. 1995; Anisfeld et al. 2017), with upland mineral soils rarely having higher than 10% organic matter (Truog 2016). The finding that *S. patens* collected from Scarborough grew equally well in upland and marsh soils could be attributed to a local adaptation of Scarborough plants to low soil organic content, and thus a tolerance to organic matter conditions similar to those of upland soil; an advantage to growing in the upland soil treatment that Biddeford and Wells plants, with a native soil organic content many times higher than the organic content of upland soil, did not have. However, differences in *S. patens* vegetative growth within the upland soil treatment suggest that additional site characteristics besides soil organic matter content could be important. For example, the growth of Biddeford plants in the upland soil treatment was midway between the growth of Scarborough and Wells plants and not significantly different from either (Fig. 2-4), even though the organic matter content at
the Biddeford site differed significantly from that at Scarborough. Further investigation into other site characteristics, such as hydrology, soil drainage class, and the availability of nutrients, would be needed to pinpoint what additional factors influence the growth of *S. patens* in upland soil. My study did not include tidal hydrology as an experimental factor because my focus was on the transition of the marsh into the upland, where *S. patens* is farthest from the ocean and should experience the least inundation. However, incorporating inundation levels that *S. patens* would experience at the upland boundary into future studies would improve our understanding of how *S. patens* plants will respond to migration because inundation has an important influence on salt marsh plant growth and zonation (Adams 1963; Broome et al. 1995). My observation that plants from different sites vary in their ability to grow in upland soil, which suggests that some *S. patens* population will find inland migration easier than others, indicates a need for further research to determine what drives this differing response to upland soil.

The presence of ground litter had no effect on *S. patens* vegetative growth variables (Tables 1-3). In contrast to my findings, and in support of my prediction for the litter treatment, other research demonstrated that the presence of litter decreased aboveground vegetative growth (Facelli and Pickett 1991; Xiong and Nilsson 1999). Differences between my results and those of other studies could be due to differences in methodology. The litter present treatment in my study was created by placing litter on top of the upland or marsh soil surrounding the *S. patens* plug (but not on top of the existing stems in the plug), while in many other studies the litter was laid directly over both initial and emerging stems (Tolley and Christian 1999; Xiong et al. 2001). The litter
treatment in my experiment, therefore, would only block the emergence of stems sprouting outside of the initial plugs, and stems rarely emerged outside of the initial plug during my study. It is possible, had the experiment extended for a second growing season, thus allowing more time for the rhizomes that grew in the first season to send up stems, that a greater difference in growth would be seen between pots containing litter and those without. Litter did significantly affect reproduction in a three-way interaction with salinity and site (Table 4), but there was no consistent pattern in how the number of pots containing flowers responded to litter and salinity among or within the three sites. Research suggests that late in the growing season temporary wrack litter disturbance to *S. alterniflora*, another salt marsh species, can decrease flowering (Li and Pennings 2017); however, few studies have addressed the effects of litter and salinity on salt marsh flowering over an entire growing season, and more research is necessary to clarify their impact on *Spartina* reproduction.

Salinity plays a critical role in defining salt marsh vegetative boundaries by constraining plant species to specific zones based on their susceptibility to salt stress (Adams 1963; Byers and Churma 2007), yet, contrary to my prediction for the salinity treatment, I did not find a significant difference in vegetative growth between the 4.5ppt and the 14.5ppt salinity treatments (Tables 1, 2, and 3). My results are consistent with those by Broome et al. (1995) who found no significant differences in the number of *S. patens* stems or aboveground biomass at five salinity levels ranging from 0 to 20ppt, but conflict with the results of Ewing et al. (1995), who observed a decrease in *S. patens* aboveground biomass at 14ppt compared to 7ppt, and Snedden et al. (2014), who
observed a decrease in belowground biomass at 8ppt compared to 3.9ppt. I suggest that these differences could be because *S. patens* populations are known to differ in susceptibility to salinity (Silander and Antonovics 1979; Pezeshki 1991; Pezeshki et al. 1993). For example, the source of the plants in Ewing et al.’s (1995) study was a brackish marsh with an approximate salinity of 2ppt. While I did not record salinity levels in the marshes in my study, measurements of salinity in the creeks within the sites ranged from 8 to 30ppt, suggesting that my plants in their native environment had a higher exposure to salt than the plants in Ewing et al.’s (1995) study, and thus, presumably, had an overall higher salt tolerance.

**Implications and Future Research**

My research indicates that some *S. patens* populations in Maine were better at growing in upland soil than others, which might translate into an advantage for inland migration, and thus could have important implications both for prioritizing areas for salt marsh conservation, and for choosing restoration plant sources. In particular, I suggest that salt marsh conservation efforts focus on preserving *S. patens* populations growing in soil with a low percentage of organic matter, because in my study the *S. patens* plants with the highest growth success in upland soil were from a site with low soil organic content. By identifying *S. patens* populations that could make the transition into upland soil without a significant decrease in growth, my study joins a growing body of research which aids in determining sites for successful inland migration (Feagin et al. 2010; Smith 2013). It is important to focus on plant populations with the highest potential to migrate
into the upland so that conservation organizations, often limited by finances, spend their resources on salt marshes that have a better chance of persisting over the long term.

Plants from *S. patens* populations that grow well in upland conditions could also be used in salt marsh restoration sites to improve the migration potential of the restored marsh. However, future research should obtain a clearer understanding of what site characteristics, including organic matter content, could influence the ability of *S. patens* to grow well in upland soil. Testing more sites across the native *S. patens* species range, from Maine south to Florida and west along the coast to Texas (Bush and Houck 2008), would provide more details on what factors influence *S. patens* migration into upland soils. By conducting a manipulative experiment, I was able to control treatment factors and limit the number of variables potentially confounding my results, but research expanding on my experiment should include field studies to confirm that my results are reproducible in a natural setting.
REFERENCES


Schieder NW, Walters DC, Kirwan ML. 2018. Massive upland to wetland conversion compensated for historical marsh loss in Chesapeake Bay, USA. Estuar Coast. 41:940–951.


Table 1: ANOVA results for the adjusted final number of stems by treatment factor. The final number of stems was adjusted by the number of initial stems per pot, the number of days the stems grew per pot, and the presence or absence of flowers.

<table>
<thead>
<tr>
<th>Treatment Factor</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>2</td>
<td>273</td>
<td>137</td>
<td>0.79</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>12811</td>
<td>12811</td>
<td>21.65*</td>
</tr>
<tr>
<td>Litter</td>
<td>1</td>
<td>1093</td>
<td>1093</td>
<td>1.85</td>
</tr>
<tr>
<td>Salinity</td>
<td>1</td>
<td>7374</td>
<td>7374</td>
<td>0.37</td>
</tr>
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<td>23211</td>
<td>11605</td>
<td>19.61*</td>
</tr>
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<td>Site x Litter</td>
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<td>2546</td>
<td>1273</td>
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</tr>
<tr>
<td>Site x Salinity</td>
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<td>282</td>
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<tr>
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<td>1</td>
<td>1</td>
<td>&lt;0.01</td>
</tr>
<tr>
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<td>1187</td>
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<td>240</td>
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<td>Site x Litter x Salinity</td>
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<td>1089</td>
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<td>0.92</td>
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<tr>
<td>Soil x Litter x Salinity</td>
<td>1</td>
<td>23</td>
<td>23</td>
<td>0.04</td>
</tr>
<tr>
<td>Site x Soil x Litter x Salinity</td>
<td>2</td>
<td>93</td>
<td>46</td>
<td>0.08</td>
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<tr>
<td>Residuals</td>
<td>88</td>
<td>52078</td>
<td>592</td>
<td></td>
</tr>
</tbody>
</table>

*p ≤ 0.05
Table 2: ANOVA results for the adjusted stem biomass by treatment factor. The stem biomass was adjusted by the number of initial stems per pot, the number of days the stems grew per pot, and the presence or absence of flowers.

<table>
<thead>
<tr>
<th>Treatment Factor</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
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<td>0.09</td>
<td>0.04</td>
<td>1.85</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>0.23</td>
<td>0.23</td>
<td>9.99*</td>
</tr>
<tr>
<td>Litter</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>Salinity</td>
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<td>0.12</td>
<td>0.12</td>
<td>1.11</td>
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<tr>
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<td>0.43</td>
<td>0.21</td>
<td>9.24*</td>
</tr>
<tr>
<td>Site x Litter</td>
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<td>0.02</td>
<td>1.17</td>
</tr>
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<td>0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>Soil x Litter</td>
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<td>0.02</td>
<td>0.02</td>
<td>0.92</td>
</tr>
<tr>
<td>Soil x Salinity</td>
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<td>0.01</td>
<td>0.01</td>
<td>0.22</td>
</tr>
<tr>
<td>Litter x Salinity</td>
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<td>0.02</td>
<td>0.02</td>
<td>0.79</td>
</tr>
<tr>
<td>Site x Soil x Litter</td>
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<td>0.04</td>
<td>1.51</td>
</tr>
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<td>0.03</td>
<td>1.45</td>
</tr>
<tr>
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<td>0.04</td>
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</tr>
<tr>
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<td>0.03</td>
<td>1.46</td>
</tr>
<tr>
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<td>0.01</td>
<td>0.01</td>
<td>0.22</td>
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<tr>
<td>Residuals</td>
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<td>2.04</td>
<td>0.023</td>
<td>0.023</td>
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</table>

*p ≤ 0.05
Table 3: ANOVA results for the adjusted rhizome biomass by treatment factor. The rhizome biomass was adjusted by the number of initial stems per pot, the number of days the rhizomes grew per pot, and the depth of the soil subsidence around each plug within the pots.

<table>
<thead>
<tr>
<th>Treatment Factor</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>2</td>
<td>0.60</td>
<td>0.30</td>
<td>2.78</td>
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<td>Soil</td>
<td>1</td>
<td>2.13</td>
<td>2.13</td>
<td>19.85*</td>
</tr>
<tr>
<td>Litter</td>
<td>1</td>
<td>0.18</td>
<td>0.18</td>
<td>0.20</td>
</tr>
<tr>
<td>Salinity</td>
<td>1</td>
<td>0.03</td>
<td>0.03</td>
<td>1.11</td>
</tr>
<tr>
<td>Site x Soil</td>
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<td>0.91</td>
<td>0.46</td>
<td>4.26*</td>
</tr>
<tr>
<td>Site x Litter</td>
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<td>0.09</td>
<td>0.05</td>
<td>0.43</td>
</tr>
<tr>
<td>Site x Salinity</td>
<td>2</td>
<td>0.13</td>
<td>0.06</td>
<td>0.60</td>
</tr>
<tr>
<td>Soil x Litter</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>Soil x Salinity</td>
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<td>0.17</td>
<td>0.17</td>
<td>1.54</td>
</tr>
<tr>
<td>Litter x Salinity</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Site x Soil x Litter</td>
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<td>0.38</td>
<td>0.19</td>
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<tr>
<td>Site x Soil x Salinity</td>
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<td>0.32</td>
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</tr>
<tr>
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<td>0.20</td>
<td>0.10</td>
<td>0.40</td>
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<tr>
<td>Soil x Litter x Salinity</td>
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<td>0.10</td>
<td>0.88</td>
</tr>
<tr>
<td>Site x Soil x Litter x Salinity</td>
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<td>0.05</td>
<td>0.02</td>
<td>0.80</td>
</tr>
<tr>
<td>Residuals</td>
<td>88</td>
<td>9.42</td>
<td>0.11</td>
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</tr>
</tbody>
</table>

\*p \leq 0.05
Table 4: Results from the poisson log-linear model of flowering by treatment factor

<table>
<thead>
<tr>
<th>Treatment Factor</th>
<th>DF</th>
<th>G Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>2</td>
<td>16.50*</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>6.49*</td>
</tr>
<tr>
<td>Litter</td>
<td>1</td>
<td>1.60</td>
</tr>
<tr>
<td>Salinity</td>
<td>1</td>
<td>0.91</td>
</tr>
<tr>
<td>Site x Soil</td>
<td>2</td>
<td>7.07*</td>
</tr>
<tr>
<td>Site x Litter</td>
<td>2</td>
<td>0.79</td>
</tr>
<tr>
<td>Site x Salinity</td>
<td>2</td>
<td>3.97</td>
</tr>
<tr>
<td>Soil x Litter</td>
<td>1</td>
<td>2.91</td>
</tr>
<tr>
<td>Soil x Salinity</td>
<td>1</td>
<td>1.94</td>
</tr>
<tr>
<td>Litter x Salinity</td>
<td>1</td>
<td>1.53</td>
</tr>
<tr>
<td>Site x Soil x Litter</td>
<td>2</td>
<td>3.83</td>
</tr>
<tr>
<td>Site x Soil x Salinity</td>
<td>2</td>
<td>1.46</td>
</tr>
<tr>
<td>Site x Litter x Salinity</td>
<td>2</td>
<td>6.54*</td>
</tr>
<tr>
<td>Soil x Litter x Salinity</td>
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<td>0.10</td>
</tr>
<tr>
<td>Site x Soil x Litter x Salinity</td>
<td>2</td>
<td>3.39</td>
</tr>
</tbody>
</table>

*p ≤ 0.05

Table 5: ANOVA results for percent soil organic matter content by site

<table>
<thead>
<tr>
<th>Treatment Factor</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>50.80*</td>
</tr>
<tr>
<td>Residuals</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

*p ≤ 0.05
Fig. 1: The map of the collection sites for *S. patens* plugs, marsh soil, and upland soil. The teardrop-shaped symbols represent, from north to south, the locations of the Scarborough, Biddeford, and Wells collection sites in southern Maine for *S. patens* and the marsh soil. The star within a circle symbol represents the upland forest soil collection site in Scarborough, ME. Map courtesy of Google My Maps (2018).
Fig. 2: The site and soil effects on average adjusted final number of *S. patens* stems (± s.e.m.). An asterisk indicates a significant difference between marsh and upland soil treatments within a collection site. Letters indicate significant differences among sites within the upland soil treatment – sites with the same letter are not significantly different from one another.
Fig. 3: The site and soil effects on average adjusted *S. patens* aboveground stem biomass (± s.e.m.). An asterisk indicates a significant difference between marsh and upland soil treatments within a collection site. Letters indicate significant differences among sites within the upland soil treatment – sites with the same letter are not significantly different from one another.
Fig. 4: The site and soil effects on average adjusted *S. patens* belowground rhizome biomass (± s.e.m.). An asterisk indicates a significant difference between marsh and upland soil treatments within a collection site. Letters indicate significant differences among sites within the upland soil treatment – sites with the same letter are not significantly different from one another.
Fig. 5: The site and soil effects on the number of pots (out of 40) containing *S. patens* flowers. The asterisk indicates a significant difference between marsh and upland soil treatments within the Biddeford collection site. Letters indicate significant differences among sites within the upland soil treatment – sites with the same letter are not significantly different from one another.
APPENDICES

APPENDIX A THE ANOVA RESULTS FOR COVARIATE TERMS

The following tables present the multiple linear regression results for the significant covariate terms and interactions used to calculate the residual response variable for the treatment factor ANOVA.

APPENDIX A I: The multiple linear regression results for the final number of stems covariate terms

<table>
<thead>
<tr>
<th>Covariate Term</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of grow days</td>
<td>1</td>
<td>22725</td>
<td>22725</td>
<td>24.02*</td>
</tr>
<tr>
<td>Flowering</td>
<td>1</td>
<td>11071</td>
<td>11071</td>
<td>11.70*</td>
</tr>
<tr>
<td>Number of initial stems</td>
<td>1</td>
<td>2830</td>
<td>2830</td>
<td>2.99</td>
</tr>
<tr>
<td>Number of grow days x Flowering</td>
<td>1</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Number of grow days x Number of initial stems</td>
<td>1</td>
<td>48652</td>
<td>48652</td>
<td>51.42*</td>
</tr>
<tr>
<td>Flowering x Number of initial stems</td>
<td>1</td>
<td>8616</td>
<td>8616</td>
<td>9.11*</td>
</tr>
<tr>
<td>Number of grow days x Flowering x Number of initial stems</td>
<td>1</td>
<td>1946</td>
<td>1946</td>
<td>2.06</td>
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</table>

*p ≤ 0.05

APPENDIX A II: The multiple linear regression results for the stem biomass covariate terms

<table>
<thead>
<tr>
<th>Covariate Term</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of grow days</td>
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<td>1.25</td>
<td>1.25</td>
<td>20.80*</td>
</tr>
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<td>Flowering</td>
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<td>3.21</td>
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<td>Number of initial stems</td>
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<tr>
<td>Number of grow days x Number of initial stems</td>
<td>1</td>
<td>2.87</td>
<td>2.87</td>
<td>18.59*</td>
</tr>
</tbody>
</table>

*p ≤ 0.05

APPENDIX A III: The multiple linear regression results for the rhizome biomass covariate terms

<table>
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<th>Covariate Term</th>
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<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Number of initial stems</td>
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<td>2.51</td>
<td>2.51</td>
<td>4.15*</td>
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<tr>
<td>Depth of soil subsidence</td>
<td>1</td>
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<td>21.17</td>
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<td>Number of grow days x Depth of soil subsidence</td>
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<td>2.36</td>
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</tr>
<tr>
<td>Number of initial stems x Depth of soil subsidence</td>
<td>1</td>
<td>9.21</td>
<td>9.21</td>
<td>15.20*</td>
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</table>

*p ≤ 0.05
APPENDIX B THE PERMITS FOR THE SCARBOROUGH COLLECTION SITE

STATE OF MAINE
DEPARTMENT OF INLAND FISHERIES AND WILDLIFE

PERMIT

ISSUED TO:
Tessa Dowling
241 Boom Rd.
Saco, ME 04074
(413) 320-3457
tdowling@une.edu

EFFECTIVE DATE
5/22/17

RENEWABLE
Yes

FEE
No

NAME OF PRINCIPAL OFFICER (if business) TYPE OF PERMIT

Scientific collection permit

LOCATION WHERE AUTHORIZED ACTIVITY MAY BE CONDUCTED –

Scarborough Marsh Wildlife Management Area

CONDITION OF PERMIT

Permittee(s) may collect plant and core samples of sediments and from marsh as part of research project with University of New England.

Permittee(s) will do their best to avoid excessive disturbance in marsh as well as avoiding sample plots from existing bird research within Scarborough Marsh WMA.

MDIFW requests copies of any publications that may arise from this research.

REPORTING REQUIREMENTS

If this research continues in successive years, please call a regional biologist (657.2345) 3 weeks in advance to organize activities.

SIGNATURE OF AUTHORIZED AGENCY REPRESENTATIVE

Brad Zitske

TITLE
Assistant Regional Wildlife Biologist

DATE
5/22/17

SIGNATURE OF AUTHORIZED AGENCY REPRESENTATIVE

Brad Zitske

TITLE

DATE
STATE OF MAINE  
DEPARTMENT OF INLAND FISHERIES AND WILDLIFE  

PERMIT

**ISSUED TO:**  
Tessa Dowling  
241 Boom Rd.  
Saco, ME 04074  
(413) 320-3457  
tdowling@une.edu

**EFFECTIVE DATE**  
4/11/18

**RENEWABLE**  
Yes

**FEE**  
No

**NAME OF PRINCIPAL OFFICER (if business)**  

**TYPE OF PERMIT**  
Scientific collection permit

**LOCATION WHERE AUTHORIZED ACTIVITY MAY BE CONDUCTED –**  
Scarborough Marsh Wildlife Management Area  
Permittee is allowed to collect 24 soil samples for study on soil differences between various marshes in state

**CONDITION OF PERMIT**  
Permittee(s) may collect plant and core samples of sediments and from marsh as part of research project with University of New England.
Permittee(s) will do their best to avoid excessive disturbance in marsh as well as avoiding sample plots from existing bird research within Scarborough Marsh WMA.
MDIFW requests copies of any publications that may arise from this research.

**REPORTING REQUIREMENTS**  
If this research continues in successive years, please call a regional biologist (657.5746) 3 weeks in advance to organize activities.

<table>
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<th>DATE</th>
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<tr>
<td>Brad Zitske</td>
<td>Assistant Regional Wildlife Biologist</td>
<td>4/11/18</td>
</tr>
</tbody>
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