Salinity Tolerance of Common Reed *(Phragmites australis)* at the Medouie Creek Restoration Site, Nantucket MA

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In 2008, the Nantucket Conservation Foundation (NCF) initiated a salt marsh restoration project at the Medouie Creek Wetland Complex, Nantucket, Massachusetts. This involved installing a box culvert under a restrictive dike road to reconnect tidal, saltwater hydrology throughout the marsh, in which the restricted portion had converted to freshwater conditions sometime in the 1930s (Karberg 2014; Figure 1). The impounded marsh hosted a large population of *Phragmites australis* (common reed) (1.58 hectares) and the re-introduction of salt water was intended to dramatically impact the population, potentially reducing the future need for herbicide treatment. Since the culvert opened in 2008, monthly soil pore water salinity monitoring has documented steadily rising salinity levels, as well as dramatic reductions in *Phragmites* patch size, stem density and stem height (Karberg 2014). The *Phragmites* population has been reduced since the restoration but not eliminated. This article examines a greenhouse study designed to determine the salinity tolerance of *Phragmites* stems collected from the restoration area.

Impacts and Ecology of Non-Native *Phragmites*

Phragmites populations can be found on all continents except Australia. In North America there exists both a native and a non-native genotype. The non-native genotype is now seen most commonly in dense, monoculture stands primarily in freshwater wetlands but occasionally in brackish and salt water wetlands (Roman et al. 1984; Saltonstall 2002). Numerous studies have documented the expansion of the non-native *Phragmites* into marsh habitats (e.g., Rice et al. 2000; Amsberry et al. 2000; Chambers et al. 1999) and the resulting decline in diversity of vegetative species, leading to habitat loss for waterfowl, shorebirds, and other wildlife (Meyerson et al. 2000). Aggressive eradication of *Phragmites* in freshwater wetlands has been shown to increase native plant diversity (Meyerson et al. 2000). Understanding how to effectively control expanding non-native *Phragmites* populations is important to maintaining the ecological integrity of many North American wetlands.

Many control mechanisms have been examined for *Phragmites* including mowing, burning, digging and herbicide application (Michigan Natural Features Inventory, no date). Each of these methods can negatively impact native species and the surrounding wetland and soil ecology. On Nantucket, NCF primarily employs direct application

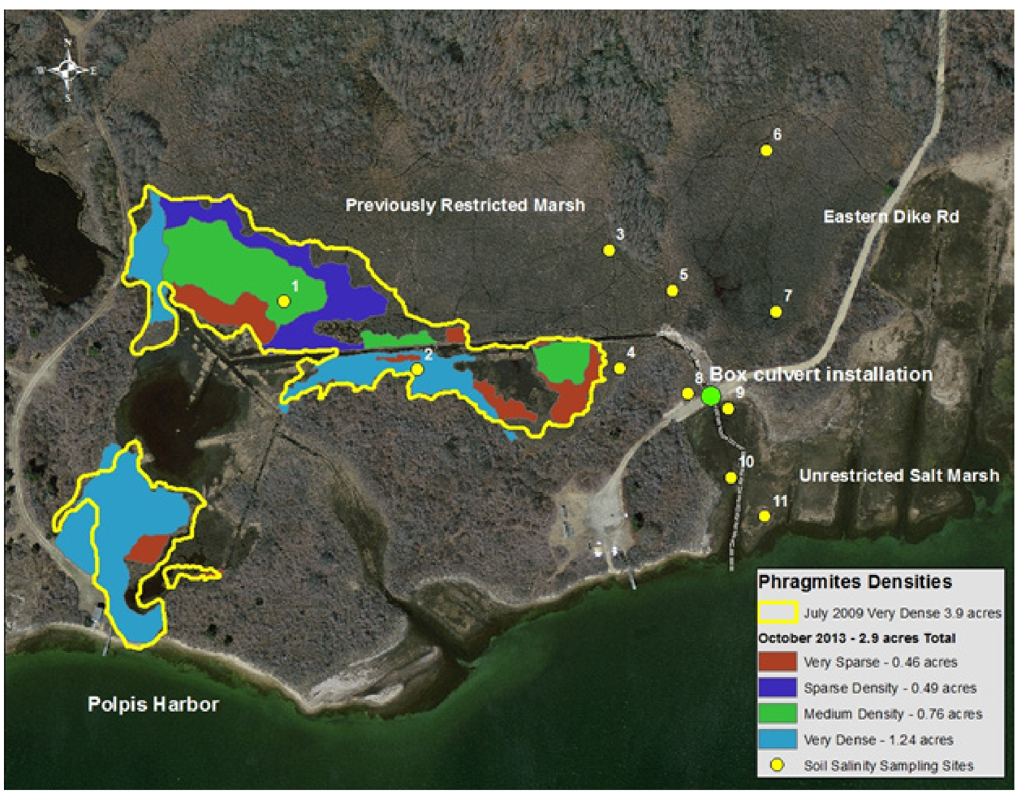

Figure 1. Medouie Creek Wetland Complex restoration area. In December 2008, a culvert was placed under the Eastern Dike Road, opening up the previously restricted marsh and reintroducing tidal flow and salinity.

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of herbicide to individual *Phragmites* stems (the cut and drip method) which can be both time and labor intensive (Omand 2014; Simmons 2013), particularly when addressing a large stand like that at Medouie Creek. A less direct method of *Phragmites* control is the introduction of salt water to marsh soils, particularly for areas that previously received saltwater inputs. Many recent studies have examined the influence of different salinity levels on the growth and production of *Phragmites* plants with varied results (Chambers et al. 2003) but they generally observed a decline in *Phragmites* with increased salinity. Lissner and Schierup (1997) saw *Phragmites* stands dieback in response to salinities higher than 15 ppt within the soil rooting zone, while their greenhouse study showed plant growth rates negatively correlated to increases in salinity. *Phragmites* morphology (e.g., height) and biomass production have been negatively correlated with increasing soil salinity levels (Hellings and Gallagher 1992) although plants collected from different locations show negative responses at different levels of salinity which may indicate a local adaptation to soil salinity levels.

Phragmites at Medouie Creek

Based on the results of previous research a restoration project was designed, attempting to reduce the population of *Phragmites* at Medouie Creek by reintroducing salt water through the installation of a culvert under the eastern dike road in 2008 (Figure 1). Overall, the population has responded negatively to increased salinity since the restoration, however, not all areas of the marsh have seen dramatic reductions in *Phragmites* populations. This may be due to differences in salinity levels throughout the marsh. Other research has shown that *Phragmites* appears to respond to increased salinity to varying degrees, possibly related to different *Phragmites* genotypes (Chambers et al. 2003; Hellings and Gallagher 1992; Lissner and Schierup 1997). On average, soil salinity has increased from approximately 0-5 ppt to 15-30 ppt across the restoration area although salinity increases have been variable across the marsh leaving some areas of *Phragmites* not as heavily impacted as others (Karberg 2014). Biennial vegetation monitoring has documented a decrease in overall *Phragmites* area from 1.58 hectares (3.9 acres) in 2008 to 1.17 hectares (2.9 acres) in 2013, as well as a decrease in *Phragmites* stem density (from ~40% to 20% within 1 m^2 plots) and height (from ~ 3.3 m to 1.26 m) (Karberg 2014) (Figure 2).

After observing declines in *Phragmites* in the field, a greenhouse study was undertaken to document the specific response of *Phragmites* stems collected from the Medouie Creek Wetland Complex to different levels of soil water salinity over a two-year growth span. Understanding the response of these local *Phragmites* plants to different salinity levels in a controlled environment will provide a better understanding of target salinity levels that need to be achieved at Medouie Creek to effectively control and/or drastically impact *Phragmites*. This information will assist in planning and prioritizing additional restoration activities at Medouie Creek in the future.

Methods

Collection of *Phragmites* Stems from Medouie Creek

In late May 2012 (Year 1), *Phragmites* rhizomes were randomly selected for hand digging at the Medouie Creek Wetland Complex and immediately placed in buckets of water and transported to the NCF research greenhouse. Stems were gently washed to remove soil and pulled apart so only one stem was present on each rhizome. Stems were planted into three gallon pots using a 2:1 potting mixture of sterile potting mix (Metro Mix® 200) and washed local sand. Up to three stems were planted in each pot to help simulate the degree of competition seen in the field (Chambers et al. 2003; Ravit et al. 2007). Pots were placed in large black

Figure 2. Photos taken in October 2008 (left) and October 2013 (right) from the exact same position within Medouie Creek showing the dramatic reduction in both height and density of Phragmites stems after the restoration. Stem heights in October 2008 averaged 3.3 m whereas heights in October 2013 averaged 1.26 m (Karberg 2014).

plastic trays, with six pots per tray. Trays were initially filled with unaltered tap water. Pots were first watered top down and then routinely watered from the bottom up by watering directly into the trays, to maintain moist soil. Pots were placed on greenhouse benches located outside on the north side of a building to provide shade and shelter from the wind.

Salinity Treatment and *Phragmites* Monitoring

Phragmites stems were allowed two weeks to equilibrate to transplanting before the application of salinity treatments. In mid-June, Instant Ocean Sea Salt® (by Instant Ocean, purchased from www.amazon.com) was mixed with tap water and applied to each tray to adjust salinity levels. A total of five trays with six pots per tray were included in this study, with each tray containing a different salinity treatment (0 ppt, 10 ppt, 20 ppt, 30 ppt, or 40 ppt) (Figure 3). Salinity was checked using a refractometer every four days. Water levels and salinity were adjusted to avoid drying out and to maintain the correct treatment levels. Trays were also visited after major rainfall events to check and adjust salinity levels.

Initial morphological measurements were taken prior to salinity application and repeated every two weeks over the course of the growing season. Morphological characters measured on each plant included stem height (mm) to the apex of the plant, basal diameter (cm), total leaf number per stem and number of dead leaves per stem. Additionally leaf length and leaf width measurements of all leaves on each stem were taken at the beginning and end of each growing season. At the end of the first growing season (October 2012) trays were placed in a fenced community garden. Trays were dug into holes in the garden soil and filled with water to allow the rhizomes to over winter without freezing. In May 2013 (Year 2), trays were removed from the community garden and again placed on greenhouse benches in the same location as in Year 1. Experimental salinity levels were reestablished for each tray and all *Phragmites* stems that emerged during the Year 2 growing season were monitored. Salinity levels and morphological measures were repeated as in Year 1.

Data Analysis

A repeated measures general linear model examined changes in each morphological characteristic between the five treatment levels in each treatment year (SPSS version 21.0) (IBM Corp 2012). Mauchly's test assessed sphericity and, depending on the value of ϵ, corrected when necessary with the Greenhouse-Geisser ($\epsilon < 0.75$) or the Huynh-Feldt ($\epsilon > 0.75$) corrections. Analysis was conducted separately for each treatment year: Year 1 (2012) and Year 2 (2013). A Bonferroni post-hoc test examined differences between salinity levels (IBM Corp 2012).

Results

During the first season of salinity treatments (Year 1), *Phragmites* stems showed significantly lower stem heights in the 30 ppt ($p \leq 0.001$) and 40 ppt ($p=0.037$) salinity treatments only as compared to the 10 ppt and significantly lower leaf numbers per stem in the 30 ppt ($p \leq 0.001$) and 40 ppt ($p=0.002$) salinity treatments as compared to the 10 ppt. No significant difference was observed in stem diameter or number of dead leaves related to salinity treatment during the Year 1 growing season.

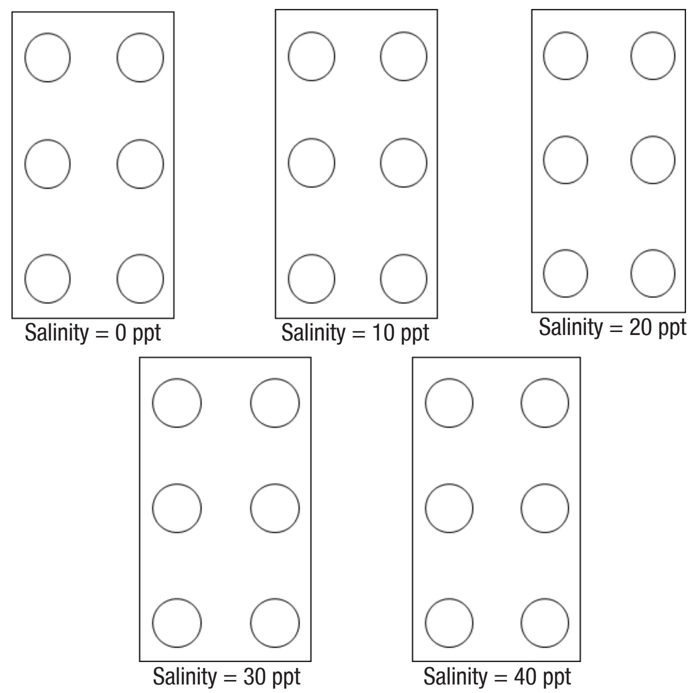

Figure 3. Experimental setup consisting of five trays, each containing six pots of Phragmites stems. Each tray was filled with water and salinity was maintained at 0, 10, 20, 30, or 40 ppt. In Year 2, each tray contained the same pots and received the same salinity treatment as in Year 1.

Figure 4. Experimental set up showing physical differences in Phragmites stems at the end of Year 2 (2013), with stems subjected to higher salinity treatments, particularly 30 ppt and 40 ppt, showing decreased stem height and robustness.

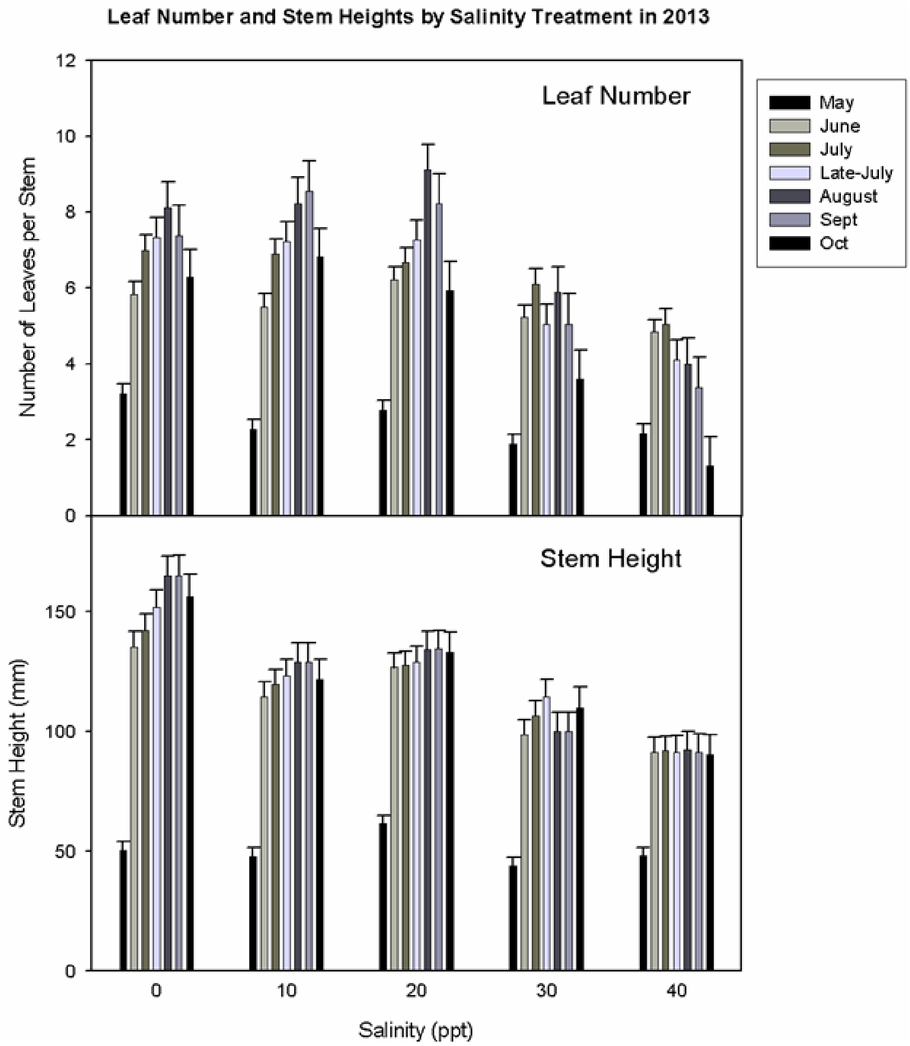

Figure 5. Leaf number and stem height in Year 2 (2013) by salinity treatment level over the course of the growing season. Plants in the 30 ppt and 40 ppt treatment had significantly less leaf production compared to the lower salinity treatments ($p<0.001$). Initial stem heights were equivalent, but heights at the lower salinity treatments quickly increased past the 30 and 40 ppt treatments ($p<0.001$).

Phragmites growth was more severely impacted by salinity treatments during Year 2 of this study. Stem heights, after an initial month of growth, decreased significantly with increased salinity, with dramatic, significant decreases seen at the 30 ppt ($p\leq0.001$) and 40 ppt ($p\leq0.001$) treatment levels (Figure 5). Leaf numbers significantly decreased in the 40 ppt treatment compared to the 0 ppt ($p\leq0.001$), 10 ppt ($p\leq0.001$) and 20 ppt ($p\leq0.001$) treatments (Figure 5). Initial stem diameters decreased significantly at 40 ppt as compared to the 0 ppt ($p\leq0.001$), 10 ppt ($p=0.049$), and 20 ppt ($p=0.010$) treatments at the start of Year 2 and did not recover over the growing season (Figure 6). The number of dead leaves significantly increased with increasing salinity levels. Overall, *Phragmites* growth and robustness significantly decreased at 30 ppt and 40 ppt after two years of exposure to elevated salinity levels (Figure 4). Morphological characters were not significantly impacted between 0-20 ppt.

Discussion

Increased Salinity Directly Impacts *Phragmites* Growth

Salinity impacts appeared cumulative over time, with stem diameters significantly smaller when exposed to high salinity levels after two years of treatment (Figure 6). The cumulative impacts of two years of exposure to higher salinity levels appeared to cause increased response in Year 2.

Phragmites has shown varying responses to increased salinity levels, potentially indicating localized adaptations to salinity tolerance (Chambers et al. 2003; Lissner and Schierup 1997). Examining the response of *Phragmites* stems collected from the Medouie Creek restoration area to increased salinity levels suggests that 30 ppt appears to be the salinity level at which, all things being equal, *Phragmites* plant health and vigor begins to dramatically decline.

Phragmites Response in the Field to Salinity

Field observations of *Phragmites* and salinity at Medouie Creek showed a response of the stems to a lower soil salinity level than that observed in this common garden experiment. Since 2008 (one year prior to restoration), our monthly sampling has shown steadily increasing salinity over time post-restoration, with salinities now averaging between 10-32 ppt for once essentially freshwater areas. Stations directly within *Phragmites* populations currently average 10-20 ppt. Despite the fact that these sites have not been exposed to the consistently high salinities that the common garden experiment suggested were needed to negatively impact *Phragmites,* significant decreases in *Phragmites* stem density, height and vigor were observed in the field (Karberg 2014). These changes could be a result of periodic pulses of high salinity due to extreme high tides, storm surge, and/or other environmental stressors that increase rhizome exposure and sensitivity to salinity. Additionally, many of the large pulses of salinity occurred during winter storms which can concentrate salinity within the soil, impacting plant growth at the start of the growing season. Although these results are encouraging, colonization by juvenile *Phragmites* stems has been observed in areas of the marsh with lower average salinities, indicating that additional management actions will likely be required in this marsh particularly as *Phragmites* shifts its distribution.

Management Considerations

This common garden experiment showed significant negative impacts to *Phragmites* stems with soil salinities of 30 ppt and higher. Current salinities observed at Medouie Creek average between 20-30 ppt but further increases in salinity appear to have leveled off. Without additional dramatic increases in soil salinity, further impacts to the current *Phragmites* population are unlikely. Lissner and Schierup (1997) observed that *Phragmites* plants with established rhizomes can exist at 22 ppt, while juvenile plants experienced high mortality which may help limit future spread of *Phragmites* throughout the marsh. Additionally, Chambers et al. (2003) reported that once *Phragmites* has effectively colonized a wetland, it can persist in soil salinities of 45 ppt and 100% inundation, although juvenile plant colonization is unlikely to occur where soil salinity is higher than 10 ppt.

The results of this study, coupled with observations of *Phragmites* at Medouie Creek suggest that soil salinity modification alone is not likely to be an effective management strategy unless the entire *Phragmites* population can be consistently exposed to adequate, increased salinity levels. At sites like Medouie Creek where the *Phragmites* population extends across a natural gradient of soil salinity levels, there will likely always remain a portion of the marsh favorable to *Phragmites*. Therefore, further management at Medouie Creek to control the *Phragmites* population could include opening up additional tidal access creeks to increase salinity throughout the marsh as well as targeted herbicide treatments to decrease and eliminate *Phragmites* located at sites exposed to lower, more tolerable salinity levels. ■

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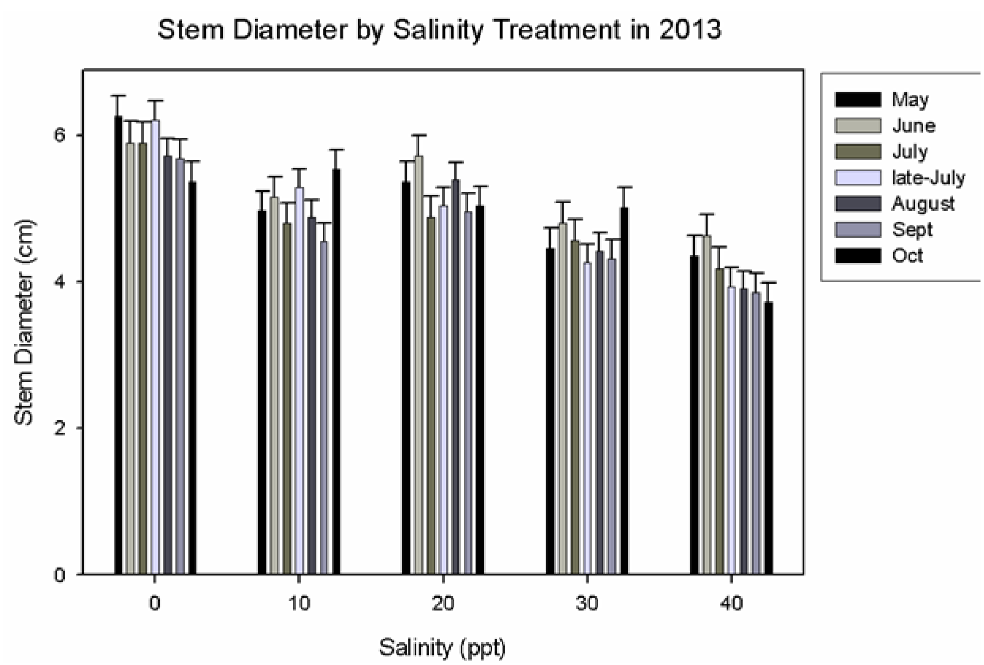

Figure 6. Stem diameters in Year 2 (2013) by salinity treatment level over the course of the growing season. Stems showed decreased diameters with increased salinity levels even at the beginning of the growing season with stems at 40 ppt much smaller compared to the 0 ppt ($p<0.001$), 10 ppt ($p=0.049$), and 20 ppt ($p=0.010$) treatments.