

Salt in the Wound: Relationship between salinity gradients, stomatal morphology, and epigenetics in the common reed, *Phragmites australis*
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Owing to a long history of human globalization, biological species invasions increasingly threaten native species diversity and ecosystem function. Among these invaders, the common reed, *Phragmites australis* (Family: Poaceae) is one of the most well-described due to its broad global distribution. In North America, *P. australis* is an invasive ecosystem engineer hailing from Eurasia that aggressively spreads into freshwater and brackish habitats. As an ecosystem engineer, it can dramatically alter local environmental conditions. It forms dense monocultures, with drastic consequences for local biodiversity and degradation of key wetland ecosystem services.

While *P. australis* thrives in human-disturbed marshes, historically it has been confined to the upper (high) marsh and terrestrial border. The high salinity of seawater and periodic tidal inundation make coastal salt marshes a particularly harsh and variable environment for any marsh plant, and are thought to be key factors limiting *P. australis* invasion. Specifically, high porewater salinity in marsh soils slows water uptake through the roots, leading to exacerbated water stress. However, rising CO₂ concentrations and nutrient pollution from terrestrial runoff have been shown to ameliorate salinity stresses and facilitate *P. australis* invasion into lower marsh areas typically dominated by the more salinity-tolerant, native cordgrass, *Spartina alterniflora*.

Stomata are microscopic pores on the surfaces of leaves that enable exchange of gases between the atmosphere and the leaf interior. They are critical in the uptake of CO₂ for photosynthesis as well as mediating plant water relations. The leaf interior is typically much more humid than the atmosphere, so water evaporates out of the leaf through the stomata in a process known as transpiration. Opening stomata, therefore, invokes a tradeoff between photosynthetic CO₂ assimilation and evaporative water loss. Plants can dynamically regulate their stomatal aperture to balance the competing needs of CO₂ uptake and water conservation. However, over longer time scales, plants also regulate stomatal development in the size and number of their stomata. How *P. australis* regulates its stomata may have important implications for its ability to invade high salinity areas, especially since *P. australis* is known to possess high phenotypic plasticity—or the ability to modify anatomical and physiological traits in response to environmental conditions. Plasticity can often be traced down to the genetic level, where differential gene expression due to external factors (epigenetics) leads to changes at the anatomical and physiological level.

Relatively few studies have explored the relationship between porewater salinity, stomatal morphology, and epigenetics in combination in *P. australis*. I collected *P. australis* leaf samples from stands in two tidal marshes in coastal Maine. I used location in the stand as a proxy for porewater salinity; that is, I assumed the terrestrial border would have a lower salinity than the leading edge of the stand. I used environmental scanning electron microscopy (ESEM) to visualize and measure stomatal characteristics (number, size) of *P. australis* from two tidal marshes in coastal Maine. I also used next generation nanopore sequencing to assess the methylation frequency (a measure of epigenetic signature of differential gene expression) of *P. australis* genes previously implicated in photosynthesis and salinity tolerance. While I did not find differences between stand edges in stomatal morphology, my results suggested that other site-specific differences can influence stand and stomatal morphology. Additionally, I found differential methylation based on the combined influences of site and stand edge, but these results highlighted the need for a fully sequenced and annotated (i.e. labeled genes) *P. australis* genome.

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