

## **Developing seed addition strategies to enhance resilience of a restored salt marsh**

**Introduction.** Accelerating rates of climate change present challenges to traditional approaches to ecosystem restoration and wetland conservation. For coastal wetlands, sea level rise (SLR) is a prominent threat that has led to subsidence of low marsh platforms and the conversion to mudflats [1]. One restoration method to mitigate this loss of wetlands is to replace sediment back onto the marsh platform to raise elevation and anticipate future years of SLR [2]. In addition to SLR, other climatic factors influence whether the sediment addition is successful in restoring coastal marsh ecosystems [1,2]. For example, further climatic shifts to warmer temperatures and lower precipitation rates will place increased stress on regions already prone to drought [1]. As a result of these climatic consequences, there is a need for further research on new restoration approaches so that we can continue restoring and protecting salt marshes.

Measures of restoration success vary from site-to-site and are determined by reference sites, stakeholder groups, and historical ecosystem services [3,4]. Resumption of natural hydrological flow is one important measure of restoration success, as well as increase in native vegetation coverage, and reintroduction of native fauna [4]. Bottlenecks - such as invasive species intrusion or failure of flora or fauna to recolonize [3] - can occur in restoration by preventing complete 'success' of established criteria. It is crucial to measure restoration success via specific measures so that findings are communicable to the restoration community.

My research addresses the bottleneck of unsuccessful vegetation colonization after sediment addition and drought, and aims to explore methodologies that promote seed additions as an efficient technique for restoring coastal wetlands. Seed additions are one of the most effective methods to revegetate large areas of land quickly and efficiently. Alternatives to seed addition based vegetation would be the use of clonal propagations from greenhouse-grown plants, which require extensive resources to implement [1], and would lack genetic diversity for a resilient marsh [5]. It is crucial to consider resiliency as a measure of success for salt marsh restoration projects, as resilience is a measure of the ecosystems ability to respond to disturbances by withstanding and recovering from damages [6]. This research will be critical as SLR and drought continue to be a prominent issue for our coastal wetlands, leading to restoration teams needing to find ways to vegetate large areas of land efficiently and quickly for successful restoration of coastal marshes.

**Background.** Hester Marsh is a coastal salt marsh found at Elkhorn Slough National Estuarine Research Reserve (ESNERR) in Monterey Bay, California. A few decades ago, the marsh - which was once important cultural grounds for the Amah Mutsun Tribal Bands - subsided into mudflats due to diking from railroads and SLR [7]. To restore the marsh ecosystem, the team at ESNERR acquired NOAA funding to place sediment back on the mudflat and raise the elevation of the marsh platform. This task began in 2016, and sediment additions are expected to be done in early 2024, with enough sediment added to anticipate SLR for 50 years and construct a high marsh platform. Because large amounts of sediment are needed to be transported and deposited,

construction equipment is used, which compacts the soil in this drought-prone region. The sediment added did not originate from marsh soil, therefore it lacks a marsh microbiome, has less organic matter, and is composed of a different chemical makeup.

There are a few strategies that could be used to combat drought and compaction (e.g., sprinkler systems, tilling the soil) as it relates to the total landscape of the salt marsh, but it is crucial to also consider revegetation efforts. *Salicornia pacifica* (pickleweed) is the dominant, native perennial plant in ESNERR salt marshes. Unfortunately, because of these stressful conditions, the vast majority of the restored lands have been unable to naturally revegetate pickleweed or other native forbs like *Frankenia salina*, *Distichlis spicata*, or *Jaumea carnosa*. Thus far, all seed additions at Hester Marsh have been unsuccessful. Freshwater irrigation, tilling of soils, and additions of soil amendments have all failed to produce any seedlings from added seed. *S. pacifica* is a pioneer species with a strong aptitude to colonize in highly saline soils - making it a strong contender for future restoration projects in estuaries [6,7]. The overall objective of this project is to develop seed addition methodology that can be utilized for large-scale coastal salt marsh restoration projects. I will use the following three questions to investigate conditions needed for germination, strategies for improving site conditions, and differences in seed quality and germination from different seed sources.

**Research Question & Hypotheses.** In this study, I will attempt to answer the following research questions:

1a. What are the main drivers of *S. pacifica* germination and seedling survival?

H1a. High moisture rates and high soil organic matter ameliorate the conditions of the soil, improving seedling germination and survival.

1b. What are the main inhibitors of *S. pacifica* germination and survival?

H1b. Soil compaction, which affects soil moisture absorption and plant growth, is the main inhibitor of seed germination and seedling survival.

H1b. Hypersalinity is a secondary inhibitor of seed germination and seedling survival.

2. What field manipulations improve germination and revegetation in salt marsh restoration projects?

H2. Introduction of microtopography will decompact soil, allow for higher moisture absorption, and increase soil-biota interactions, improving germination and revegetation.

H2. The use of cover crop as a facilitator to increase nitrates rates will increase nutrient amounts and improve germination rates.

H2. The use of the priorly-existing marsh mud will increase nutrient levels to improve germination rates.

3. How does plant provenance and seed origin affect *S. pacifica* seed germination and survival?

H3. Seeds that originate in areas with conditions that best replicate site-specific stressors will best be adapted to germinate and survive.

**Methods. Question 1.** My first question is a comprehensive analysis on *Salicornia pacifica* through its early life history. I will use viability tests on the seeds in the lab, greenhouse experiments in both CA and FL, and extensive literature research on *S. pacifica* to address this question. Lab experiments take place in petri dishes using soil directly from Hester as a substrate, or taking place with no substrate for baseline viability measurements. Viability trials consist of 50 seeds per petri dish, 3 replicates per seed source, and I will count the number of seeds germinated every 3 days for 2 weeks [8]. Germination occurs when the radicle is extended out from the seed [9]. Viability tests will be replicated every month for 8 months following harvest of pickleweed seeds to determine age of maximum viability of the seeds.

Greenhouse experiments in Florida will take place from January to early May, when outdoor temperatures are closer to current central California weather. Greenhouse experiments focus on the success of germination and survival with several treatments: soil amendments (coir, fertilizer, potting soil, and manure), stressor conditions (moisture, compaction, organic rates, and salinity), and scarification techniques (soaking or not). In order to determine primary inhibitors of *S. pacifica*, I will establish a full factorial experiment with gradients of compaction, moisture and salinity (high, medium or low), under high or low organic soil substrates (potting soil or hester soil), and then record the number of germinants. Florida greenhouse experiments are then replicated in California during field visits to corroborate results.

**Question 2.** My second question determines what kinds of large-scale field manipulations are successful in improving revegetation and reestablishment of seeds. I will look at the use of cover crop as a facilitator for marsh plant colonization and a nitrogen fixer for improved soil conditions, microtopography as a way to ameliorate current soil conditions, and inoculation of marsh microbes - through channel slurry/mud and reference marsh root balls - to promote plant-soil biota interactions. The use of cover crop as a tool for coastal restoration is untested in marsh restoration, but could theoretically decompact and improve nutrient levels in the soil [10]. Alternatively, the use of existing channel mud from nearby reference sites have been tested and data shows it could build the microbial community and increase organic matter to improve soil conditions [11,12]. Both cover crop and channel mud/root balls are upcoming methods to test in late 2024/early 2025 and will be tested by adding those respective organic sources to meter squared plots in conjunction with seed additions. Microtopography experiments were established by tilling the large areas of bare soil and through building runnels off water channels and creeks. These areas should hold more water and trap seeds coming in on the rising tide. Off of a large creek channel, I established 10 meter-long runnels with a gradual slope leading from channel edge into the high marsh [13]. Natural recruitment in and around the runnels will be monitored through aerial imagery (UAV; vegetation indices) and cover of plants in vegetation plots. Upcoming seed additions will be tested in conjunction with these microtopography types.

**Question 3.** Lastly, seed provenance (quality of seeds from different source areas) has been long established as critical to restoration efforts [4,14], but have rarely been studied in coastal wetland settings. I will test seed quality by establishing an index of stressor and environmental conditions across source areas - compaction, inundation frequency, elevation, and organic matter (e.g. high

inundation frequency, medium compaction, low elevation, and high organic matter rates). Pickleweeds seeds will be collected at 10 sites and 2 harvest dates. Each seed source will be tested for baseline viability - through previously described seed viability methods - to determine seed quality differences. They will then be planted in the greenhouse to determine if the seeds express germination plasticity in healthy or stressful soil conditions. I will simulate benign conditions through high watering frequencies with high nutrients in potting soil, with stressful conditions being the reverse in Hester's low organic soil. Trials will track not only germination, but will follow plants through the first 3 months of establishment to track any seedling mortality.

**Progress.** I was brought onto this project in January 2023 as a Masters student who was only focusing on the greenhouse experiments in my first question. Since converting to a PhD program in November of 2023, I have greatly expanded the scope of the project. None of the seed source experiments have begun, nor have any large-scale seed additions taken place yet. Microtopography has been established with no monitoring points conducted, and cover crop and channel mud amendments have not taken place yet.

**Broader Impacts.** My research will be disseminated through three different avenues. First off, as part of adaptive management efforts, throughout my research process, I will be sharing results with land managers at ESNERR so they can incorporate my findings to their large-scale restoration efforts and plans at Hester for the last phase of construction and restoration. In addition to this, I have already presented my findings and plans to a group restoration practitioners, and plan to continue to present my annual findings to keep them updated. Next, my research will be shared through UF/IFAS Electronic Data Information Source (EDIS) documents in order to reach a non-scientific public audience. I am currently working with Florida Sea Grant agents on outreach EDIS documents surrounding mangrove migration and management. I will use this experience to write another document on wetland restoration and conservation in a changing climate. This research project exemplifies the need for wetland intervention and restoration, and demonstrates the realities that global warming has on our coastal ecosystems. This knowledge is critical for public audiences in the face of climate change rates accelerating. Lastly, I plan to present the results of these studies at national conferences, such as the Coastal and Estuarine Research Federation, in addition to publishing through journal articles.

In order to conserve coastal wetlands, we must be adaptive to future climatic changes, such as SLR and increased frequencies of episodic drought. Restoration techniques like the ones used at Hester, of replacing sediment onto the subsiding and sunken marsh platform, need to be further tested and monitored for its success in restoring coastal wetlands. There has already been established knowledge that replacing the marsh platform can lead to self-sustaining and resilient marshes [15]. By researching ways to build on this success, we can prevent future bottlenecks that negate vegetation colonization on these coastal wetlands.

**Use of Funds.** Funds will be utilized primarily for travel expenses to the field site. Each trip I take to my field site guarantees more trials for seed additions, additional monitoring points for accurate vegetation establishment and growth rates, and extra time for making crucial observations while the field site is still in the early stages of vegetation establishment.

### Works Cited

1. Bertolini, C. and da Mosto, J. (2021), Restoring for the climate: a review of coastal wetland restoration research in the last 30 years. *Restor Ecol*, 29: e13438.
2. Raposa, K. B., Bradley, M., Chaffee, C., Ernst, N., Ferguson, W., Kutcher, T. E., ... & Wigand, C. (2022). Laying it on thick: Ecosystem effects of sediment placement on a microtidal Rhode Island salt marsh. *Frontiers in Environmental Science*, 10, 939870.
3. Zedler, J. B. (2000). Progress in wetland restoration ecology. *Trends in ecology & evolution*, 15(10), 402-407.
4. Wortley, L., Hero, J. M., & Howes, M. (2013). Evaluating ecological restoration success: a review of the literature. *Restoration ecology*, 21(5), 537-543.
5. Reynolds, L. K., McGlathery, K. J., & Waycott, M. (2012). Genetic diversity enhances restoration success by augmenting ecosystem services. *PloS one*, 7(6), e38397.
6. Cahoon, D. R., McKee, K. L., & Morris, J. T. (2021). How plants influence resilience of salt marsh and mangrove wetlands to sea-level rise. *Estuaries and Coasts*, 44(4), 883-898.
7. Fountain, M., Jeppesen, R., Endris, C., Woolfolk, A., Watson, E., Aiello, I., Fork, S., Haskins, J., Beheshti, K., Wasson, K. Hester Marsh Restoration. Annual Report 2021. Elkhorn Slough National Estuarine Research Reserve. Available <https://www.elkhornslough.org/tidal-wetland-program/>.
8. Baskin, C. C., & Baskin, J. M. (2014). *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. Elsevier/AP.
9. Elsey-Quirk, T., Middleton, B. A., & Proffitt, C. E. (2009). Seed flotation and germination of salt marsh plants: the effects of stratification, salinity, and/or inundation regime. *Aquatic Botany*, 91(1), 40-46.
10. Qi, G., Chen, S., Ke, L., Ma, G., & Zhao, X. (2020). Cover crops restore declining soil properties and suppress bacterial wilt by regulating rhizosphere bacterial communities and improving soil nutrient contents. *Microbiological Research*, 238, 126505.
11. Adam, P. (2019). Salt marsh restoration. In *Coastal wetlands* (pp. 817-861). Elsevier.
12. Baptist, M. J., Gerkema, T., Van Prooijen, B. C., Van Maren, D. S., Van Regteren, M., ... & van Puijenbroek, M. E. B. (2019). Beneficial use of dredged sediment to enhance salt marsh development by applying a 'Mud Motor'. *Ecological Engineering*, 127, 312-323.
13. Watson, E. B., Ferguson, W., Champlin, L. K., White, J. D., Ernst, N., Sylla, H. A., ... & Wigand, C. (2022). Runnels mitigate marsh drowning in microtidal salt marshes. *Frontiers in Environmental Science*, 10, 2125.
14. Vander Mijnsbrugge, K., Bischoff, A., & Smith, B. (2010). A question of origin: where and how to collect seed for ecological restoration. *Basic and Applied Ecology*, 11(4), 300-311.
15. Thorne, K. M., Freeman, C. M., Rosencranz, J. A., Ganju, N. K., & Guntenspergen, G. R. (2019). Thin-layer sediment addition to an existing salt marsh to combat sea-level rise and improve endangered species habitat in California, USA. *Ecological Engineering*, 136, 197-208.