**Local adaptation in a changing world: Characterizing ecotypes across New England saltmarsh grasses**

**Statement of Work**: Saltmarshes are highly productive intertidal wetlands which provide essential ecosystem services to coastal communities such as long-term carbon sequestration (Macreadie et al., 2021; Mcleod et al., 2011), hurricane mitigation (Narayan et al., 2017), and nutrient cycling (de Groot et al., 2012); they also serve as habitat for juvenile fish (zu Ermgassen et al., 2021), migratory birds (Hanson & Shriver, 2006), endangered diamondback terrapins (Lovich et al., 2001), and unique coastal forbs like saltmarsh aster (*Symphyotrichum subulatum*), glasswort (*Salicornia spp*), and sea lavender (*Limonium carolinianum*) (Richards et al., 2005). However, saltmarshes in New England are vulnerable to sea-level rise (SLR), where SLR is ~3-4 times the global average and outpacing sediment accretion (Boon, 2012; Raposa et al., 2017a; Sallenger et al., 2012). Saltmarshes in densely populated coastal communities such as Narragansett Bay are trapped between rising oceans and human infrastructure with few opportunities for upland migration (Bradley et al., 2023; CRMC, 2015), exacerbating saltmarsh loss due to human development (Bromberg & Bertness, 2005; Gedan et al., 2009).

Loss of local saltmarsh habitat is of concern not only because of biodiversity declines and reduced ecosystem services to coastal communities, but because of potential extirpation of site-specific adaptations. Though species may have a wide geographic range, individual populations can evolve specific adaptations which confer fitness advantages in the conditions of their local habitat; these locally adapted populations are known as *ecotypes* (Turreson, 1925). When ecotypes are reduced in population size, undergo habitat destruction, or are extirpated from a region entirely, evolutionary potential can be diminished, decreasing resilience to future disturbances through the loss of locally adapted traits and genetic diversity (Jump et al., 2009; Kramer & Haven, 2009; Hufford & Mazer, 2003; Zambrano et al., 2019). Therefore, protecting native ecotypes and using local source populations for revegetation projects is an important consideration in coastal wetland conservation (Breed et al., 2018; Montalvo et al, 1997).

However, the geographic scale at which ecotypes differentiate varies by species and region (Knapp et al, 1997), and thus habitat managers in New England require region- and species-specific data to know where unique ecotypes are located, whether some ecotypes are more vulnerable to SLR than others, and how ecotype ranges may vary by species. Most saltmarsh ecotype research has been conducted on the low-marsh engineer *Spartina alterniflora*, which is differentiated genetically and in the amplitude of functional traits such as biomass, stem height, and salinity tolerance across Gulf and Atlantic populations (Blum et al., 2007; Hester et al., 1998; Seliskar et al., 2002; Travis & Grace, 2010). However, data is scarce for New England salt marsh ecotypes of *S. alterniflora* and even more rare for high-marsh grasses such as *S. patens* and *D. spicata*. These high-marsh species are among the first to be undergo habitat loss due to sea level rise (Raposa et al., 2017b) making ecotype studies for plants in this habitat zone critical to restoration and management.

To characterize regional ecotypes for saltmarsh grasses under ambient and SLR-simulated conditions and determine geographic extent for ecotypes of each species, this study will test the following hypotheses for *S. alterniflora, S. patens*, and *D. spicata* populations across New England: 1) Saltmarsh ecotypes can be identified by trait and genetic differences among populations along a latitudinal gradient; 2) Geographic range of saltmarsh ecotypes are not identical among different saltmarsh species. The data from this study will inform New England marsh prioritization for adaptive management by identifying populations vulnerable to SLR and areas of propagule sourcing for the restoration of degraded marshes.

**Work Completed**: In the summer of 2023, I collected rhizomes of three species (15 individuals per species; 45 rhizomes total per site) from nine field sites in New England ranging from Connecticut to Maine (Fig. 1). Rhizomes were propagated at URI. Clonal replicates from this collection will be used in garden experiments and DNA analysis.

**Proposed Work:** *Laboratory:* In 2024, I will identify genotypes and genetic differences among populations by extracting DNA from leaf tissue samples grown in the greenhouse (n=15 per population). DNA will be amplified via PCR using microsatellite markers developed for *S. alterniflora* (Blum et al., 2004), *S. patens* (Baisakh et al., 2009), and *D. spicata* (Tsuyusko et al., 2007); 405 samples will be processed with additional replicates. PCR products will be visualized using capillary electrophoresis on a genetic



*Figure 1. New England saltmarsh collection sites*

analyzer (Agilent 2100 Bioanalyzer) through URI's (INBRE) core facility. I will use Genodive 3.0 (Meirmans, 2020) to calculate genotypic and allelic richness, heterozygosity, genetic distance, principal component analysis (PCA), and Analysis of Molecular Variance (AMOVA); R package Genepop V1.2.2 (Roussett, 2008) will be used to calculate $F_{ST}$, $F_{IT}$, and deviations from Hardy-Weinberg equilibrium among populations. *Greenhouse*: I will identify trait differences among populations by growing plants from each population in a common garden experiment under ambient and simulated SLR conditions. Ten individuals from each population will be grown in three salinities, three flooding depths, and a combination of salinity and flooding; five clonal replicates from each individual will be used in each treatment (Table 1). Mortality, stem height, and stem density will be recorded for plants in each treatment through the summer (Hughes, 2014), and aboveground biomass, seed count, and seed mass in the fall (Liu et al., 2020) (Table 2).
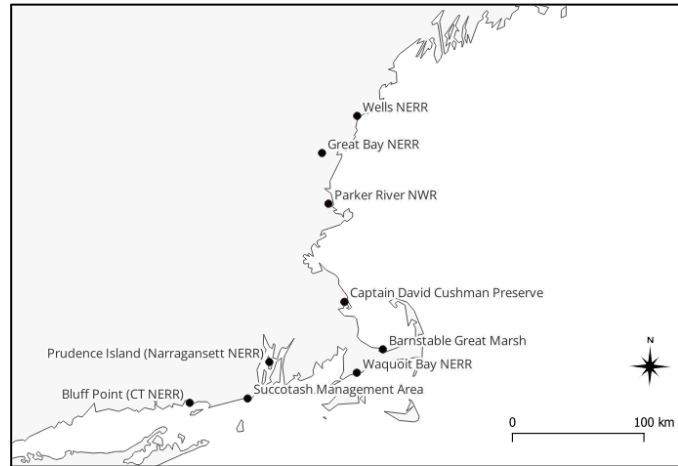
*Table 1. Common garden experiment: Salinity, flooding, and salinity x flooding treatments. Ten individuals from each of the 27 populations (3 species x 9 sites) will be used in each treatment. Each treatment will have 5 clonal replicates of each individual.*

|  | *Salinity (control = 0 psu)* | *Salinity (15 psu)* | *Salinity (30 psu)* |
|---|---|---|---|
| *Flooding (control= watering, no flooding)* | Control | Salinity only (15 psu) | Salinity only (30 psu) |
| *Flooding (saturated = water 0 cm above soil)* | Flooding only (0 cm) | Salinity (15 psu) x Flooding (0 cm) | Salinity (30 psu) x Flooding (0 cm) |
| *Flooding (water 15 cm above soil)* | Flooding only (15 cm) | Salinity (15 psu) x Flooding (15 cm) | Salinity (30 psu) x Flooding (15 cm) |

*Field sites:* In fall 2024, I will assess the degree to which traits observed in the common garden are expressed in wild source populations by returning to collection sites and measuring stem height, stem density, aboveground biomass, seed count, and seed mass along the original collection transect using point-sampling (Table 2). Trait measurements will be compared

between field and greenhouse plants for each population using a one-way analysis of variance (ANOVA) to determine the extent to which trait expression observed in the greenhouse is influenced by environmental conditions.

Table 2. Quantitative traits measured in the common garden [1] and field [2]. Superscript numbers indicate where measurements will be taken

| Growth | Stem height [1,2], stem density [1,2], aboveground biomass [1,2] |
| Fitness | Seed count [1,2], seed mass [1,2], mortality [1] |

Genetic and trait differences among populations will be compared using general linear mixed modeling (GLMM) and models assessed for best fit using Akaike Information Criterion (AIC) to determine which genetic and trait variables vary among populations. Principle component analysis using both genetic and trait data will be used to identify where populations cluster into ecotypes. Salinity and tidal datum for each collection site will be taken from NOAA monitoring datasets and compared to genetic and trait data using GLMM to determine what associations exist between population traits, genetic differences, and environmental parameters.

**Benefit to Coastal Wetlands:** This study will identify and map new saltmarsh ecotypes in a region of increased coastal wetland loss and assess the response of different ecotypes to salinity and flooding stress to identify populations at increased extirpation risk to SLR. It will compare the geographic extent of ecotypes among three dominant saltmarsh grasses in order to determine whether areas of propagule sourcing for one species may be used to inform propagule sourcing for the others, and explore how environmental conditions at field sites may have selected for ecotype traits in order to set the groundwork for future experiments. Applications of this data include adaptive management prioritization for coastal marshes, saltmarsh restoration, propagule sourcing for both high- and low-marsh revegetation, and conservation of native wetland plant species.

**Use of Funds:**

*Supplies provided by URI and previous funding:* greenhouse space, tables, hoses, water, pots, potting soil, tubs for flooding treatments, plant tags, aquarium grade dissolving sea salt

*Funds requested for genetic analyses, lab consumables, and additional greenhouse supplies:* five Omega Biotek Plant DNA extraction kits (96 preps) @ $206 each (= **$1,030**); lab consumables (plastic bags, markers, PCR plates, pipette tips, Taq, microsatellite fluorescent labels, Sybr Safe stain, TAE, vials, gloves, storage boxes, labels, tape, etc.) (=**$1,000**); Sanger sequence fragment analysis of 810 samples (replicates included) at the Rhode Island INBRE Molecular Informatics Core (MIC) @ $3.44 per sample (=**$2,786.40**); greenhouse supplies and plant care (supplies to build additional tables, plant tags, pots, etc.) (=**$180**). *Total funds requested:* **$4997.**

**Sharing Research:** End users of this data include non-profit conservation organizations, restoration practitioners, state and federal partner organizations, and coastal townships. Results will be made widely available through peer reviewed publications, conference presentations, public outreach through collaborating organizations, and technical report contributions.

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