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SHORT COMMUNICATION

LIGHT AND SALINITY EFFECTS ON *VALLISNERIA AMERICANA* SEED GERMINATION AND SEEDLING GROWTH[§]

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INTRODUCTION

Vallisneria americana Michaux is a cosmopolitan species of submerged aquatic vegetation (SAV) found in the freshwater and low salinity reaches of Gulf of Mexico (GOM) estuaries (Doering et al. 1999, Hauxwell et al. 2007, Poirrier et al. 2010, Martin and Valentine 2019, DeMarco et al. 2022). Like many SAV species, *V. americana* plays a key role in maintaining aquatic biodiversity, stabilizing sediments, improving water quality, and serving as habitat (Korshgen and Green 1988, Dennison et al. 1993, McFarland and Schafer 2008, Looby et al. 2019). *Vallisneria americana* can temporarily persist in salinities of 15—20, although salinity tolerance varies by population (Tootoonchi et al. 2020). In dynamic estuaries, salinity stress coupled with increasing anthropogenic and natural disturbances threaten this important habitat type, triggering management efforts to conserve and restore this key foundation species.

Vallisneria americana has been used as a candidate species in restoration (Korschgen and Green 1988), using methods ranging from planting individual ramets, transplanting buds, or using seed pods and seed—based practices (Moore et al. 2010, Rohal et al. 2021). Despite successful restoration in some areas, the southern US population of *V. americana* varies in numerous ways from its northern US counterparts (Rohal et al. 2024). Indeed, the southern population is often referred to as *V. neotropicalis* (Les et al. 2008), although the Integrated Taxonomic Information System does not formally recognize *V. neotropicalis* as a species and thus we retain the currently recognized *V. americana* nomenclature (Kauth and Biber 2014). Northern *V. americana* produces overwintering tubers/turions and senesces during the winter, while southern populations may persist year—round (McFarland and Shafer 2008). Reproductively, umbellate inflorescences have been documented in, and may be exclusive to, the southern population (Rohal et al. 2022). In northern *V. americana*, cold stratification improves germination rates (Jarvis and Moore 2008), however, Kauth and Biber (2014) found that was not the case with southern *V. americana* and that scarification was more effective in stimulating germination.

Given the increased interest from governmental and non—governmental organizations in restoring oligo— and mesohaline habitat types such as *V. americana*, understanding the optimal conditions for southern *V. americana* propagation, including seed germination and early life history development such as seedling growth, is of paramount importance for ecological restoration efforts. Here, we present the results of 2 experiments designed to: 1) evaluate germination differences of desiccated and non—desiccated seeds across common estuarine salinities; and 2) determine the impact of shaded/unshaded conditions at these same salinity levels on growth of recently germinated *V. americana* seedlings. Our overarching goal is to determine optimal conditions for the early life stages of *V. americana* and provide applied, practical insights that can guide conservation efforts to restore, protect, and manage these critical habitats.

MATERIALS AND METHODS

In Experiment 1, we tested whether seed germination rates differed among desiccated/non—desiccated seeds and across salinities. Seed pods were obtained from multiple locations in the Mobile—Tensaw Delta (USA) and cold—stratified over one winter in a refrigerator at 3°C. Prior to exposure to treatments, seeds were triple rinsed with 90% ethanol and deionized water for 3 min to reduce contamination (Seeliger et al. 1984, Kauth and Biber 2015). Desiccated seeds were allowed to air dry for 48 hours while non—desiccated seeds remained submerged. Seeds were then separated into individual petri dishes based on desiccation (desiccated, non—desiccated) and salinity (0, 5, or 10) treatments, with 50 seeds per replicate. These salinities mimic the location that plants were collected from and are typical for this estuary, with lower salinities (0—5) persisting most of the year with occasional peaks (10—15+) in late fall months (Martin and Valentine 2014, Kauffman et al. 2018). Each unique desiccation x salinity treatment had 5 replicates and we

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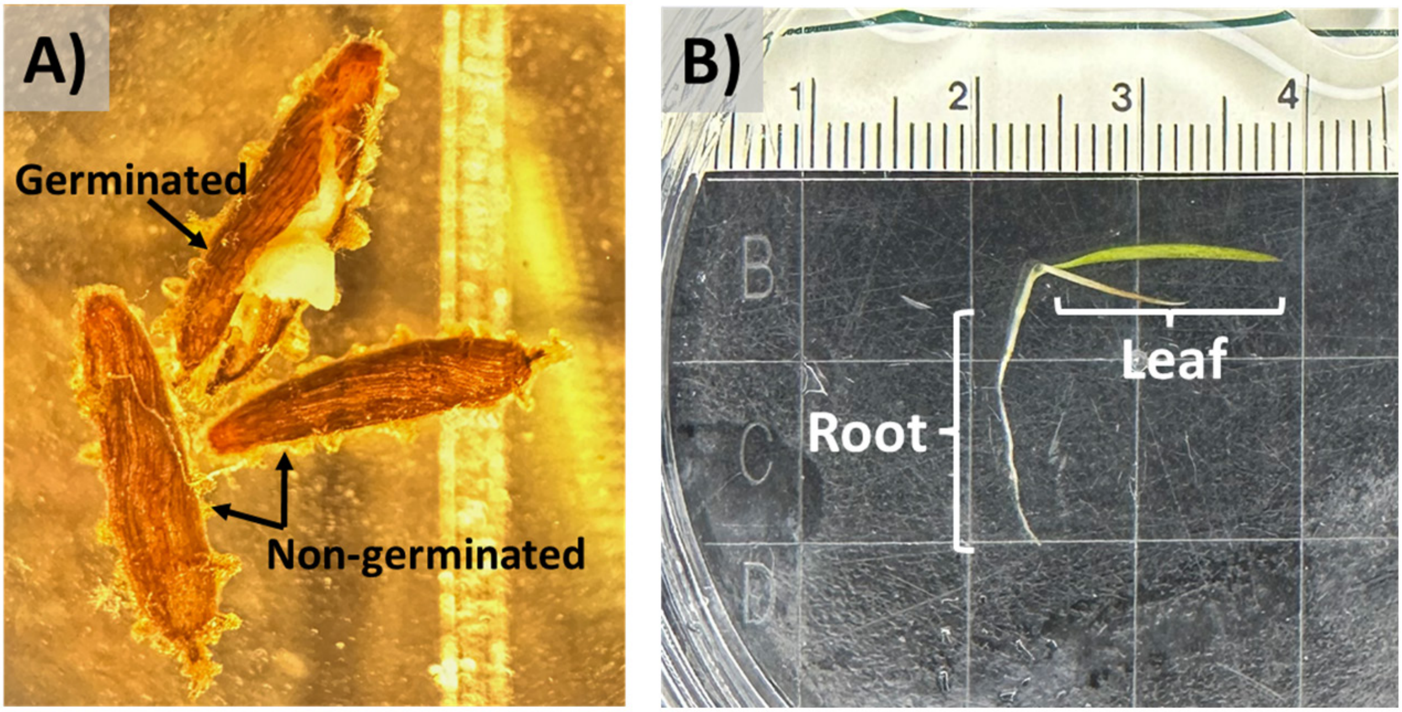

FIGURE 1. *Examples of* Vallisneria americana *germination. A. Germinated and non–germinated seeds. B. Recently germinated seedlings containing root and leaf segments.*

visually monitored for seed germination under 2.25x magnification at increasing time intervals (Figure 1A). We checked for germination after 1, 3, 5, 10, 24, 48, and 72 h after the initiation of the experiment. Because non–desiccated seeds did not germinate over this interval, we monitored their germination rates over an additional, longer period (168 hours).

In Experiment 2, we documented the effect of light and salinity on seedling root and leaf growth using recently emerged, lab–germinated seeds exposed to either shaded/unshaded conditions and various salinities (0, 5, or 10) for a 2 week period. All seedlings were randomly assigned to individual 266 mL containers and exposed to either low (shaded) or high (unshaded) light conditions at one of 3 salinity treatments (0, 5, 10), with 5 replicates per unique light*salinity treatment. Gardening shade cloth covered treatments in the shaded treatment and a HOBO TidBiT logger verified that unshaded and shaded light conditions were 867.85 + 59.27 lum/ft^2 and 51.06 + 3.00 lum/ft^2 (mean + 1 se), respectively, a reduction of ~95% of surface irradiance. Initial and final measurements of leaf (from the meristem to the end of the longest leaf) and root (from the meristem to the end of the longest root) length (Figure 1B) were taken from before/after photographs that included a ruler for scale using ImageJ software (Schneider et al. 2012).

Statistical Analyses

In Experiment 1, we compared the germination rates as proportion of seeds germinated across desiccation (2 levels: desiccated and non–desiccated) and salinity (3 levels: 0, 5, 10) treatments using a two–way Analysis of Variance. In Experiment 2, we analyzed the proportion change in leaf and root length using 2–way Analysis of Variance across light (2 levels: shaded, unshaded) and salinity (3 levels: 0, 5, or 10) treatments. Assumptions were tested prior to analyses and all data were considered significant at $p < 0.05$. When significant differences were detected, Tukey's pairwise comparisons were used to determine where significant differences occurred. All analyses were done using SigmaPlot software (https://systatsoftware.com/product/sigmaplot–v15/).

Results

In Experiment 1, germination was highly influenced by desiccation ($F_{2,25}$= 18.600; $p < 0.001$), but not salinity ($F_{2,25}$= 0.095; $p = 0.910$). Germination rates were 4 times higher in desiccated seeds (mean 0.214 $\pm$ 0.03 se) than non–desiccated seeds (mean 0.048 $\pm$ 0.012 se), despite the longer time period for germination in non–desiccated seeds (168 hours vs. 72 hours) (Figure 2A). Germination of desiccated seeds generally occurred within the first 24 hours (Figure 2B).

Growth of seedlings varied depending on salinity and light treatments in Experiment 2. Root length (Figure 3A) varied across salinities ($F_{2,24}$ = 9.63; $p = 0.001$) and light ($F_{1,24}$ = 6.77; $p = 0.016$) treatments, with no significant interaction ($F_{2,24}$ = 0.30; $p = 0.740$). Root growth was best in the 0 salinity and unshaded light treatment, with an increase of approximately 62% in root length over the duration of the experiment. In general, the 0 salinity treatment exhibited higher root growth than salinity treatment levels of 5 (T = –3.10; $p = 0.013$) or 10 (T = –4.282; $p = 0.001$) while 5 and 10 salinity trials were not significantly different (T = –1.22; $p = 0.451$). However, seedlings exposed to higher light (i.e., unshaded treatment), regard-

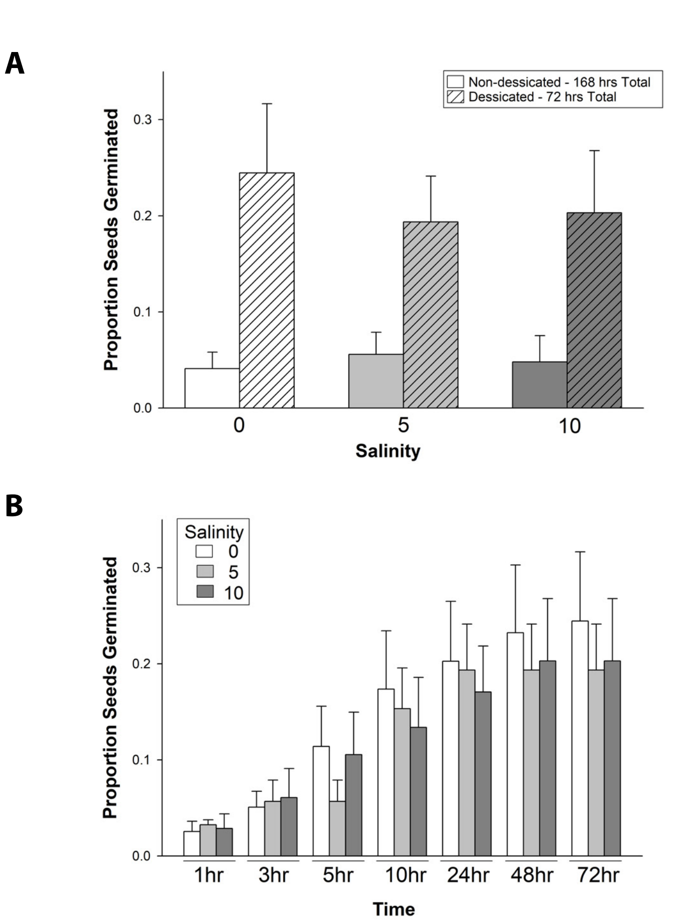

FIGURE 2. Vallisneria americana *seed germination by salinity and time. A. Mean (± se) desiccated (dashed bars) and non—desiccated (solid bars) seed germination across salinities. B. Timeline of seed germination. Most seeds germinated within 24—48 h.*

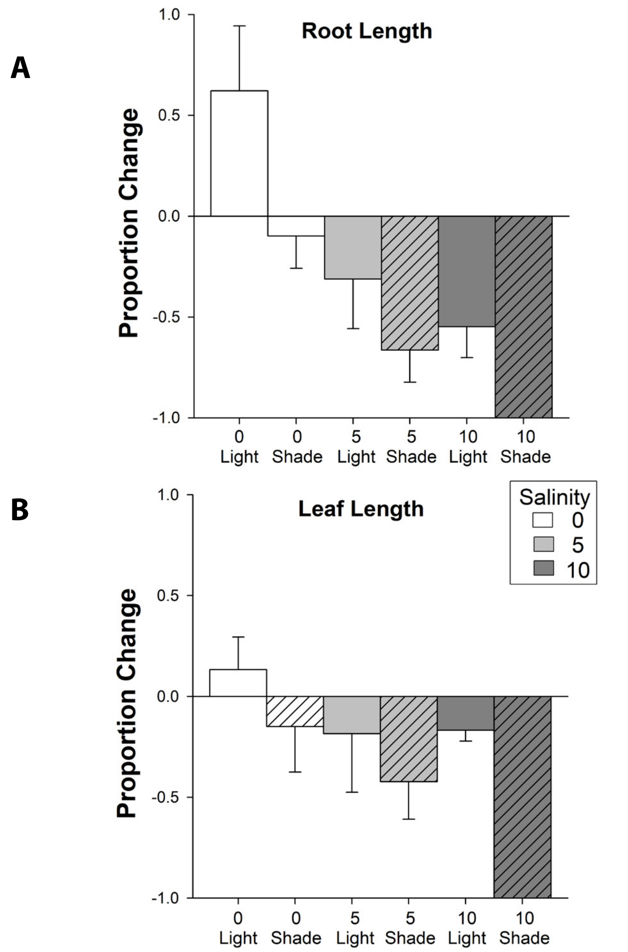

FIGURE 3. *Root and leaf growth of* Vallisneria americana*. A. Mean (± se) proportion change in seedling root lengths in light (solid bars) and shaded (dashed bars) conditions across varying salinities. B. Mean (± se) proportion change in seedling leaf lengths in light (solid bars) and shaded (dashed bars) conditions across varying salinities.*

less of salinity, tended to have longer roots than in lower light conditions (i.e., shaded) treatment (T = —2.60, p = 0.016). Leaf length similarly differed among salinity ($F_{2,24}$ = 5.11; p = 0.014) and light ($F_{1,24}$ = 9.64; p = 0.005) treatments (Figure 3B), with no interaction ($F_{2,24}$ = 1.78; p = 0.190). Again, the 0 salinity and unshaded light treatment exhibited highest growth (~10% increase) over the duration of the experiment. Pairwise comparisons indicate that the proportion of change in leaf length was not significantly different between 0 and 5 salinities (T = —1.64; p = 0.248), but did differ between 0 and 10 (T = —3.20; p = 0.010). Leaf change did not differ between the 5 and 10 treatments (T = —1.61; p = 0.26). Similar to the changes in root length, leaf length increase in the unshaded treatment was greater than in the shaded treatment (T = —3.11; p = 0.005).

Discussion

Globally, a decline in estuarine habitats such as SAV (Orth et al. 2006, Waycott et al. 2009, Dunic et al. 2021) has triggered conservation efforts to improve environmental conditions and reduce further declines. Restoration activities have gained recent attention to combat ongoing losses (Jarvis and Moore 2008, McFarland and Shafer 2008, Paice et al. 2016, Rohal et al. 2021), with numerous techniques employed depending on location. These include the harvesting of seed pods from native populations for creation of a nursery stock (Cao and Ruan 2015) which can be more effective and cost—efficient once favorable conditions at the restoration site exist (Jarvis and Moore 2008, Rohal et al. 2024). Here, we provide practical information on optimal germination and growth conditions to improve planting protocols to restore *V. americana* and the ecosystem services it provides.

In the northern GOM, the dominant estuarine SAV *V. americana* is frequently exposed to seasonal increases in saline conditions when low rainfall conditions and dominant south winds occur (Martin and Valentine 2012). Increases in salinity have been shown to negatively influence flowering (French and Moore 2003) and seed germination (Jarvis and Moore 2008) by hindering water uptake by seeds and disrupting ion balance, thereby negatively affecting germination processes (Wahid et al. 2009, Xia et al. 2020), particularly when salinity levels exceeded a certain threshold (French and Moore 2003, Jarvis and Moore 2008). We found that desiccation was more important than salinity at the levels we tested in determining germination rates for this population, findings similar to previous studies in this area (Clark 1999). In submerged vegetation, desiccation can promote germination by breaking seed dormancy and enhancing the release of germination inhibitors, similar to scarification noted in previous studies (Clark 1999, Kauth and Biber 2014, Rohal et al. 2024). Desiccation promotes water imbibition by seeds, initiating the germination process and thus may be an underrepresented adaptation that allows submerged vegetation seeds to exploit the fluctuations in water levels and successfully germinate in aquatic habitats. Although our germination rates following dessication were relatively low (~25%), these germination rates were typical compared to previous studies (Rohal et al. 2024). Our results suggest that desiccation as a pretreatment for *V. americana* seeds can enhance germination success compared to non–desiccated seeds, by a fourfold increase, and is a low–cost, effective means to induce and increase germination success instead of scarification, which can be tedious and imprecise for large scale restoration efforts (Clark 1999, Kauth and Biber 2014, Rohal et al. 2024). That said, a more quantitative evaluation of seed water loss may identify optimal levels of desiccation for future restorations.

Light and salinity are 2 key environmental factors that significantly influence *V. americana* seedling growth. Seedlings may experience reduced light conditions if they grow in existing beds or areas of reduced water clarity as well as variable salinities depending on prevailing conditions (e.g., decreased freshwater input or wind) that favor salt wedge penetration into oligohaline GOM estuaries (Martin and Valentine 2012, 2014). We found favorable growth in the lowest salinities indicating potential sensitivity to salinity stress at early growth stages. In addition, we highlight that higher light and increased water clarity promotes better seedling growth, indicating that shallower depths and less turbid areas may be targeted for restoration.

As this ecologically vital species continues to decline, information on best practices for germination and growth strategies can inform conservation, habitat management, and restoration initiatives. Here, we suggest that practitioners may enhance success by using a desiccation period prior to casting seeds and targeting lower salinity areas with restoration efforts. Restoration of natural hydrology may improve salinity conditions by reducing the variability in salinity in upper estuarine reaches (Rozas et al. 2013), further facilitating success. Future studies should determine the influence of local conditions on maternal or seed phenotypic variation and the ecological implications of seed germination under desiccated conditions. In conclusion, this work provides critical insights into this key foundation species and improves our understanding of the ecological requirements of GOM *V. americana*, including the range of conditions it can persist in. Our results provide critical information for improving restoration and preservation strategies in areas prone to multiple natural and anthropogenic disturbances such as estuaries.

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