



Tolerance and avoidance: Two contrasting physiological responses to salt stress in mature marsh halophytes *Juncus roemerianus* Scheele and *Spartina alterniflora* Loisel

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ABSTRACT

For most plants, elevated salinities can promote both hyperionic and hyperosmotic stress, often resulting in decreased growth and increased mortality. In previous studies involving plant–water relations, two contrasting physiological mechanisms to water stress have emerged: (i) stress-tolerance, which can be achieved through osmotic adjustment and changes in tissue elasticity, and (ii) stress-avoidance, which restricts further water loss through decreased stomatal conductance and changes in leaf morphology and/or orientation. While these processes have been well characterized in angiosperms during drought, few studies have considered these responses in halophytes during salt-stress. In this study, experimental microcosms were used to evaluate salt-tolerance and salt-avoidance in two contrasting coastal-marsh halophytes, *Juncus roemerianus* and *Spartina alterniflora*. In mature *S. alterniflora*, preacclimated to freshwater, only salt-tolerance mechanisms (osmotic adjustment and increased tissue rigidity) were observed during high salinity conditions. In contrast, physiological modifications observed in mature *J. roemerianus* involved salt-avoidance through decreased stomatal conductance. These physiological responses are consistent with zonation patterns in these plants, wherein *S. alterniflora* resides in the lower marsh and must contend with long-term salt exposure and *J. roemerianus* inhabits the upper reaches of salt-marshes where salinities tend to be lower and where salt-stress often involves transient exposure to high salinities.

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1. Introduction

In coastal wetlands, salinity stress plays an important role in shaping large-scale vegetation patterns that promote well-defined plant communities such as tidal-fresh, brackish, and salt marshes (Hester et al., 2001; Pennings et al., 2005). Even within systems, monotypic stands of individual plant species often reflect the heterogenetic patterns of environmental salinities, as well as the intraspecific variations in competitive ability and salt tolerance among species (Hinde, 1954; Adams, 1963; Pennings et al., 2005). Despite the importance of salinity in shaping the composition of coastal plant communities, our understanding of how different species respond physiologically to variable salinities is limited. Studies on plant responses to sudden and dynamic changes in salinity,

however, may help explain observed plant distributional patterns and abundances in coastal wetlands, as well as provide a framework on how physiological adaptations shape intraspecific variations to salt stress in coastal plant species.

Two of the more common halophytes that reside in salt marshes of the Atlantic and Gulf Coasts of North America are *Spartina alterniflora* Loisel and *Juncus roemerianus* Scheele. The perennial C₄ grass, *S. alterniflora*, is considered one of more important plant species along the eastern coast of North America, and despite its comparatively high salt resistance, higher salinities often inhibit plant growth and productivity (Phleger, 1971; Mooring et al., 1971; Broome et al., 1975). The C₃ rush, *J. roemerianus*, is typically found in monotypic stands along mid-to-upper marshes that continue upland toward terrestrial systems or as isolated patches along the shorelines of brackish waters (Reimold et al., 1973; Woerner and Hackney, 1997; Touchette, 2006). While both species have demonstrated considerable resistance to high salinities, due to their respective positions within salt marshes, it is generally accepted that *S. alterniflora* is more tolerant than *J. roemerianus* (Wiegert and Freeman, 1990; Pennings et al., 2005).

Except for differences in concentration and type of osmotica used in plant tissues (ions are more prevalent in halophytes); physiological responses in plants to salt stress are remarkably similar to those employed during drought (Kirst, 1989; Touchette, 2007). For plants with limited water availability, physiological adjustments often

Abbreviations: ALT, alternating salinity and freshwater treatment; *g*, leaf conductance; *P*–*V*, pressure–volume; *P*, turgor pressure; *P*^{sat}, turgor pressure at water saturation; SAL, constant salinity treatment; *W*_{dt}, dry weight; *W*_f, fresh weight; *W*_t, turgid weight; *ε*, bulk modulus of elasticity; *θ*, relative water content; *θ*_{sym}, symplastic water content; *θ*_{tlp}, water content at turgor loss point; *Ψ*, water potential; *Ψ*_{leaf}, leaf water potential; *Ψ*_{xylem}, xylem potential; *Ψ*_p, turgor pressure; *Ψ*_π, osmotic potential; *Ψ*_π^{sat}, osmotic potential at full saturation; *Ψ*_π^{tlp}, osmotic potential at turgor loss point.

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involve avoidance and tolerance, with most plants using some combination of the two (Morgan, 1984; Yue et al., 2006; Romanello et al., 2008). Avoidance responses may include increases in stomatal and cuticular resistance, changes in leaf morphology, and/or changes in leaf orientation (Jones and Corlett, 1992; Zlatev, 2005). In contrast, tolerance to drought stress involves maintaining adequate cell turgor while minimizing metabolic disruptions. Two of the primary mechanisms that contribute to tolerance are, (i) changes in tissue elasticity (i.e., bulk elastic modulus, ϵ), and (ii) osmotic adjustment involving inorganic ions, carbohydrates, and organic acids (including compatible solutes; Munns, 1988; Savé et al., 1993; Touchette et al., 2007; Munns and Tester, 2008).

Clearly, tolerance in the form of osmotic adjustment plays an important role in halophytes residing in saline environments (Flowers and Colmer, 2008). For water to flow through the soil–plant–atmosphere continuum, a gradient of decreasing water potentials (Ψ) must be established. The Ψ of pure water is defined as 0 MPa, increasing salinity, or concentrations of other solutes, will decrease Ψ . Thus, any sharp rise in salinity could effectively hold water osmotically away from plants that lack any physiological or morphological modifications (Larcher, 2003). To limit restrictions on water uptake, plants must generate increasingly lower Ψ to allow continued water flux into belowground structures. This is often achieved through salt tolerance, where solute concentrations within the plant (including compatible solutes) are increased (Flowers et al., 1977; Touchette, 2007; Flowers and Colmer, 2008). Without adequate modifications in salt tolerance, plants could ‘shutdown’ certain aspects of plant–water relations, and rely mostly on salt avoidance to endure brief periods of salt stress.

In comparison to agriculturally important plant species, little is known about how plant–water relations are altered in salt marsh halophytes following rapid and substantial changes in soil–water salinities. Therefore the objectives of this research were to determine short-term physiological responses to abrupt salinity stress and to identify important mechanisms of salt tolerance and avoidance in *S. alterniflora* and *J. roemerianus*. As salt challenges in coastal marshes can involve long-term salt exposure (Scavia et al., 2002) or shorter pulsed exposures (Howard and Mendelssohn, 1999; Touchette, 2006; Forbes et al., 2008), we considered both extended salt stress (up to 8 weeks) and cyclic salt stress (alternating weekly between fresh and saline conditions) in mature plants previously adapted to freshwater only. While it was expected that salt stress would alter plant–water relations in these angiosperms, our focus was to discern both similarities and differences in the physiological mechanisms that promote salt resistance in these two contrasting salt marsh species.

2. Material and methods

2.1. Culture conditions

Mature nursery-grown *J. roemerianus* Scheele and the short form of *S. Loisel* (both species planted from seeds and receiving only freshwater for more than a year) were transplanted in 20 L microcosms and maintained in greenhouse conditions (with ambient light and photoperiod, temperature between 25 and 30 °C, and relative humidity between 34 and 89%). At the start of the experiment, plants were fully mature with heights between 60 and 85 cm and total biomass of 1.3 ± 0.4 and 0.89 ± 0.3 g dry weight plant⁻¹ for *J. roemerianus* and *S. alterniflora*, respectively. Each microcosm contained approximately 12 cm of natural aquatic sediments (sandy-loam soil texture; 68% sand, 28% silt, and 4% clay) that exhibited typical redoximorphic features (i.e., soil mottling with a low chroma gleyed matrix color) of high-marsh freshwater soils. Prior to the initiation of the study, plants were maintained in flooded soils (freshwater level between 1 and 2 cm above the substratum) at a density of approximately 100 shoots m⁻² for 4 weeks to allow

acclimation to culture conditions. Treatments were initiated by draining surface- and pore-waters from experimental microcosms and replacing them with artificial seawater (Instant Ocean®, Spectrum Brands, Inc., Atlanta, GA) until a final surface water salinity of 30 psu was achieved. Salinity treatments included constant 30 psu (SAL; $n = 5$ microcosms) and alternating weekly between freshwater and 30 psu (ALT; wherein seawater was applied on even weeks; $n = 5$) over a period of 8 weeks. Controls ($n = 5$) were maintained in pre-treatment conditions (i.e., 5 cm freshwater; 0 psu).

2.2. Growth measurements

Plant biomass (aboveground, belowground, and total) and leaf area were recorded prior to the initiation of the treatments and at the end of the experiment (8 weeks). Plant biomass was determined by haphazardly removing a single plant from each microcosm, sorting the plant into above- and belowground tissue, and drying the material to constant weight at 60 °C. Prior to drying leaves, total leaf area was determined for each individual plant using a digital leaf area meter (model CI-202. CID Inc, Camas, Washington).

2.3. Plant–water relations

Weekly tissue water content (θ) in leaves was evaluated on a single plant from each microcosm according to Joly (1985) following the equation:

$$\theta = (W_f - W_d) / (W_t - W_d)$$

where W_f was the fresh weight, W_t was the turgid weight, and W_d was the oven dry weight (60 °C; $n = 5$). Turgid weight was determined by placing individual leaves in 50 mL vials containing de-ionized water, and allowing tissues to reach full turgor in darkness.

Xylem potentials (Ψ_{xylem}) were conducted on young-fully-extended leaves that were enclosed in foil bags for more than 2 h to allow for xylem tissue equilibration as described by Meinzer et al., 2001. After equilibrating, a Scholander pressure chamber (Model 1000, PMS Instrument Co., Albany, Oregon, USA) was used to determine Ψ_{xylem} . Similarly, leaf water potentials (Ψ_{leaf}) were determined using a Scholander pressure chamber on non-enclosed leaves measured between 1 and 2 h after solar noon.

A steady-state diffusion porometer (model SC-1; Decagon Devices, Pullman, WA) that measures the vapor flux between the leaf surface and the atmosphere was used to evaluate stomatal conductance (g). To account for diurnal variability in g , weekly porometer measurements (week 8 was not recorded) from plants in individual microcosms were recorded at three time intervals between 11:00 and 15:00 h. The three readings were pooled into a daily mean, and statistically reported as a single g to avoid replication within experimental unit (i.e., pseudoreplication).

Pressure–volume curves (P – V curves), as described by Tyree and Hammel (1972), were constructed by plotting the reciprocal of Ψ_{leaf} against θ ($n = 5$). Regressions on the linear portion of the curve were equivalent to tissue osmotic potential (Ψ_{π}) and were used to derive osmotic potential at full saturation (Ψ_{π}^{sat}), osmotic potential at turgor loss point (Ψ_{π}^{tlp}), water content at turgor loss point (θ_{tlp}), and symplastic water content (θ_{sym}) (Tyree and Hammel, 1972). Bulk modulus of elasticity (ϵ) was calculated from the initial portion of the curve, following Ψ_{π} correction, as described in the following equation:

$$\epsilon = \frac{d\Psi_p}{d\theta_{\text{sym}}}$$

where changes in turgor potential (Ψ_p) were compared against changes in θ , and symplastic water content (θ_{sym} ; Koide et al., 1989).

2.4. Data analysis

A general linear model (GLM) with a repeated-measures design (week sampled as the repeated-measures factor) was performed on serial data including biomass and leaf area, as well as log-transformed Ψ_{leaf} , Ψ_{xylem} , g , and θ . Post-hoc comparisons involved a Holm–Sidak pairwise multiple comparison test. Non-serial data, including physiological parameters derived from P – V curves were analyzed using a Kruskal–Wallis one-way ANOVA on ranks followed by a Tukey test for post-hoc evaluation. All comparisons were considered significant at an α of 0.05.

3. Results

Throughout the study, there were no observed mortalities or tissue damage attributed to experimental treatments. That is, we did not observe any change in leaf chlorosis or necrosis in either the controls or salt treated plants. Over the course of the eight-week study, there were no significant differences in biomass production or total leaf area in *S. alterniflora* (Table 1). Similarly, with the exception of an appreciable increase in aboveground biomass for ALT plants ($p=0.039$), no significant biomass or leaf area responses were observed in *J. roemerianus* (Table 2). Although there were trends of increased biomass and leaf area for *J. roemerianus* under most growing conditions, the apparent high variability among plants after eight weeks may have limited our ability to detect significance. This may have been further complicated by using mature plants, in comparison to seedlings, as growth rates typically wane in older plants.

Relative water content (θ) remained fairly stable in *S. alterniflora*, with no significant treatment responses (Fig. 1A; $p=0.326$). The mean θ for all plants was 93.3% of full saturation, with a minimum and maximum observed θ being 88.5 ± 2.3 and $98.7 \pm 0.8\%$, respectively. In contrast, there were significant decreases in θ for treated *J. roemerianus* by the second week (Fig. 1B; $p<0.001$). In this study, the overall θ for control *J. roemerianus* was $89.7 \pm 2.3\%$, however for treated plants θ declined to 64.6 ± 5.4 and $59.7 \pm 3.7\%$ for ALT and SAL, respectively.

As expected, both plants responded to salt treatments with appreciable decreases in water potential (both Ψ_{leaf} and Ψ_{xylem} ; Figs. 2 and 3). In *S. alterniflora* grown in control conditions, midday Ψ_{leaf} appeared somewhat stable, although there was a trend of increased

Table 1

Morphological measurements reported on *Spartina alterniflora* at the beginning (Initial) and at the end (Final) of the study for control, alternating salinity (ALT), and constant salinity (SAL).

Parameter (period)	Control	ALT	SAL
Aboveground biomass (g DWt)			
Initial	0.343 ± 0.060	0.341 ± 0.054	0.357 ± 0.053
Final	0.318 ± 0.033	0.390 ± 0.063	0.373 ± 0.070
Belowground biomass (g DWt)			
Initial	0.581 ± 0.203	0.507 ± 0.096	0.530 ± 0.124
Final	0.444 ± 0.046	0.931 ± 0.216	0.496 ± 0.075
Total biomass (g DWt)			
Initial	0.924 ± 0.261	0.848 ± 0.062	0.887 ± 0.160
Final	0.761 ± 0.062	1.321 ± 0.273	0.869 ± 0.098
Above: Below ratio			
Initial	0.949 ± 0.333	0.892 ± 0.348	0.792 ± 0.165
Final	0.748 ± 0.112	0.460 ± 0.059	0.840 ± 0.196
Leaf area (cm ² plant ⁻¹)			
Initial	29.6 ± 7.3	25.3 ± 3.1	23.9 ± 3.9
Final	26.4 ± 3.5	35.4 ± 6.3	38.1 ± 6.7
Mass per leaf area (g m ⁻²)			
Initial	154.9 ± 19.3	131.2 ± 13.9	161.3 ± 39.1
Final	124.5 ± 7.8	124.1 ± 12.3	98.8 ± 9.7

Parameters include, biomass (aboveground, belowground, and total), Aboveground: belowground ratios (Above: Below), total leaf area, and mass per leaf area. Data are presented as means ± 1 SE.

Table 2

Morphological measurements reported on *Juncus roemerianus* at the beginning (Initial) and at the end (Final) of the study for control, alternating salinity (ALT), and constant salinity (SAL).

Parameter (period)	Control	ALT	SAL
Aboveground biomass (g DWt)			
Initial	0.594 ± 0.163	0.686 ± 0.180	0.665 ± 0.225
Final	0.803 ± 0.224	1.658 ± 0.483	0.986 ± 0.267
Belowground biomass (g DWt)			
Initial	0.709 ± 0.112	0.654 ± 0.126	0.689 ± 0.295
Final	0.861 ± 0.284	0.919 ± 0.234	0.890 ± 0.158
Total biomass (g DWt)			
Initial	1.303 ± 0.267	1.339 ± 0.241	1.354 ± 0.501
Final	1.665 ± 0.451	2.578 ± 0.633	1.876 ± 0.319
Above: Below ratio			
Initial	0.763 ± 0.161	0.932 ± 0.305	0.781 ± 0.143
Final	1.064 ± 0.323	1.949 ± 0.697	1.189 ± 0.346
Leaf area (cm ² plant ⁻¹)			
Initial	19.7 ± 3.7	22.5 ± 5.0	21.9 ± 7.9
Final	37.1 ± 11.8	41.33 ± 13.7	20.7 ± 5.6
Mass per leaf area (g m ⁻²)			
Initial	272.6 ± 44.0	293.5 ± 55.5	297.6 ± 33.9
Final	217.8 ± 23.08	415.1 ± 93.6	579.8 ± 209.4

Parameters include, biomass (aboveground, belowground, and total), Aboveground: belowground ratios (Above: Below), total leaf area, and mass per leaf area. Data are presented as means ± 1 SE. Significant difference with time is in bold.

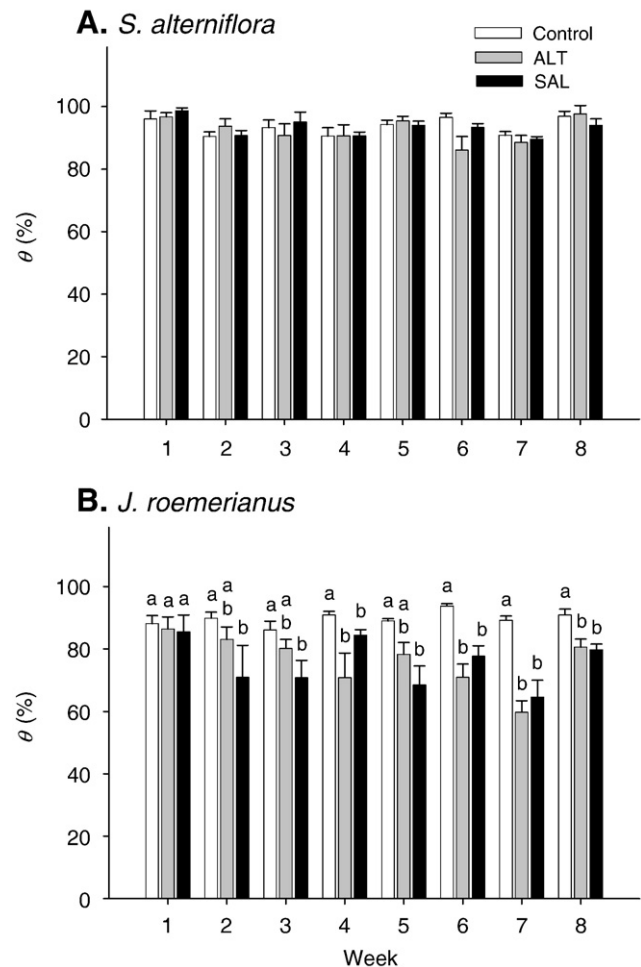


Fig. 1. Relative water content (θ ; percent of full saturation) for *S. alterniflora* (panel A) and *J. roemerianus* (panel B) receiving only freshwater (Control; white bars), alternating saltwater and freshwater weekly (ALT; seawater applied on even weeks), and only saltwater (SAL). Data are presented as means ± 1 SE, and significant differences (only observed in *J. roemerianus*) for each week are indicated by letters above the bars.

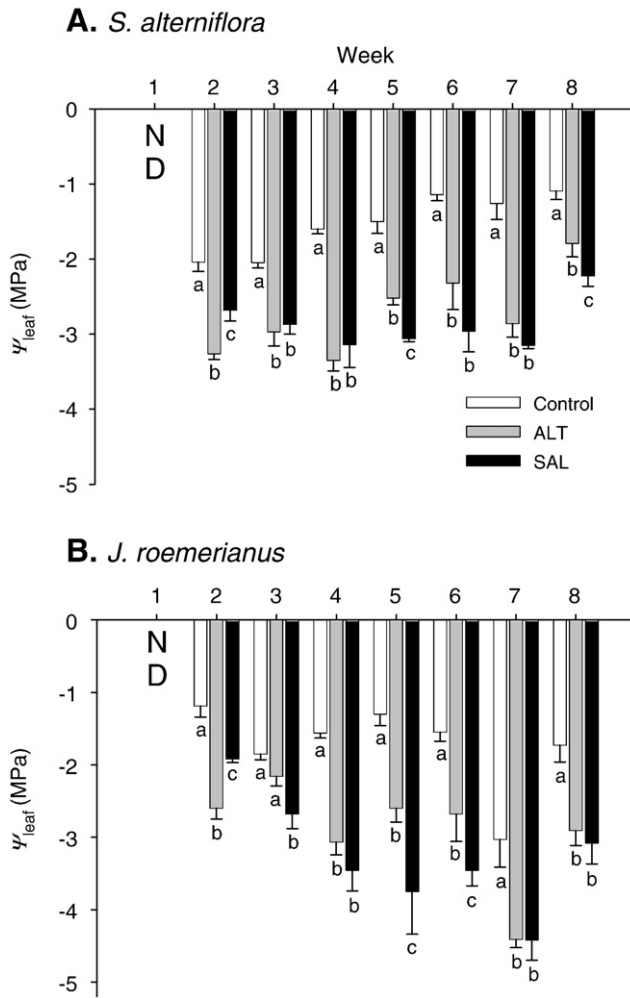


Fig. 2. Midday leaf water potentials (Ψ_{leaf} ; MPa) for *S. alterniflora* (panel A) and *J. roemerianus* (panel B) receiving only freshwater (Control; white bars), alternating saltwater and freshwater weekly (ALT; seawater applied on even weeks), and only saltwater (SAL). Data are presented as means ± 1 SE, and significant differences for each week are indicated by letters above the bars.

Ψ_{leaf} over the course of the eight-week study (from -2.0 ± 0.12 to -1.1 ± 0.11 MPa; Fig. 2A). In salt treated *S. alterniflora*, however, there were substantial decreases in Ψ_{leaf} throughout the investigation ($p < 0.001$) with overall means of -2.8 ± 0.12 and -2.7 ± 0.19 MPa for ALT and SAL treated plants. Although Ψ_{leaf} in control *J. roemerianus* were more variable over time (ranging between -1.2 ± 0.11 and -3.0 ± 0.38 MPa; Fig. 2B), significant differences from salt treatments were also noted ($p < 0.001$). The overall Ψ_{leaf} for ALT and SAL treatments in *J. roemerianus* were -2.6 ± 0.25 and -2.8 ± 0.28 MPa, however these plants did achieve the lowest recorded Ψ_{leaf} approaching -4.5 MPa on week seven (Fig. 2B). The patterns of Ψ_{xylem} for both *S. alterniflora* and *J. roemerianus* were remarkably similar to Ψ_{leaf} , although the values of Ψ_{xylem} were higher, as was expected (Fig. 3). As with Ψ_{leaf} , there were significant declines in Ψ_{xylem} for salt treated plants in *S. alterniflora* ($p < 0.001$) and *J. roemerianus* ($p < 0.001$). In control *S. alterniflora*, Ψ_{xylem} was often greater than -1.4 MPa, whereas Ψ_{xylem} in SAL treated plants were well below -1.6 MPa. While Ψ_{xylem} in ALT treated *S. alterniflora* was also lower than the controls, the decreases in Ψ_{xylem} were delayed by three weeks. Similarly, Ψ_{xylem} in control *J. roemerianus* were above -1.1 MPa, whereas SAL treated plants were typically less than -1.5 (Fig. 3B). Moreover, as with *S. alterniflora*, there was a three week delay in lowering of Ψ_{xylem} in ALT treated plants.

Stomatal conductance (g) in response to salt treatments was markedly different between the two halophytes (Fig. 4). The daily

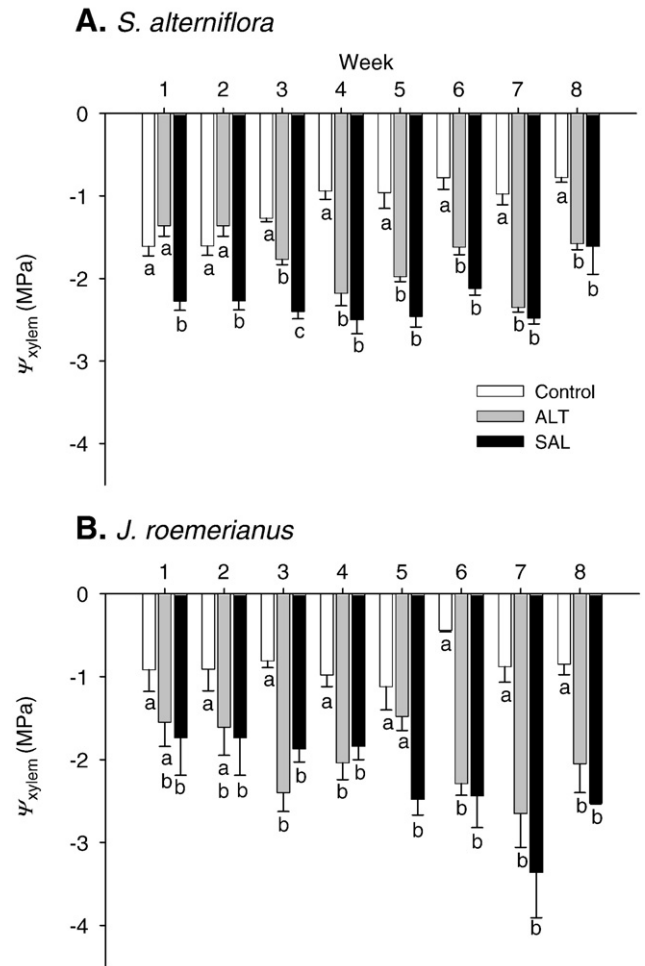


Fig. 3. Predawn xylem potentials (Ψ_{xylem} ; MPa) for *S. alterniflora* (panel A) and *J. roemerianus* (panel B) receiving only freshwater (Control; white bars), alternating saltwater and freshwater weekly (ALT; seawater applied on even weeks), and only saltwater (SAL). Data are presented as means ± 1 SE, and significant differences for each week are indicated by letters above the bars.

mean g in *S. alterniflora* was highly variable over different sampling dates (between 9.2 ± 1.8 and 42.4 ± 2.0 mmol H₂O m⁻² s⁻¹; Fig. 4A). This observed temporal variability may be attributed, in part, to ambient light, temperature, and humidity conditions at the time g was measured. Nevertheless, as these conditions were unique for each sample period, comparisons between the control and treatments for each individual day can provide valuable insight in how g responded to different treatments. On week 1, a significant difference was observed between ALT and SAL treated plants ($p = 0.017$), wherein g in SAL was nearly two-fold higher than ALT. In contrast, by the third week there was a significant decrease in g for salt treated *J. roemerianus* ($p < 0.001$), which appeared to decline further as the study progressed (Fig. 4B). By the seventh week, g was 52.2 ± 17.4 mmol H₂O m⁻² s⁻¹ in control plants but had declined to only 17.4 ± 5.5 and 14.3 ± 1.5 mmol H₂O m⁻² s⁻¹ for ALT and SAL plants, respectively.

Changes in plant–water relations, as determined through pressure–volume analyses, were observed at the end of the study for *S. alterniflora* (Table 3). While there were no significant differences in symplastic water fraction (θ_{sym} ; $p = 0.48$) between the control and treatments, there were significant decreases in osmotic potential at turgor loss point (Ψ_{π}^{tlp}) for both ALT and SAL ($p = 0.009$), as well as a decrease in osmotic potential at saturation (Ψ_{π}^{sat} ; $p = 0.025$), and an increase in turgor pressure at saturation (P^{sat} ; $p = 0.025$) for SAL treated *S. alterniflora* (Table 3). Furthermore, there was a three-fold

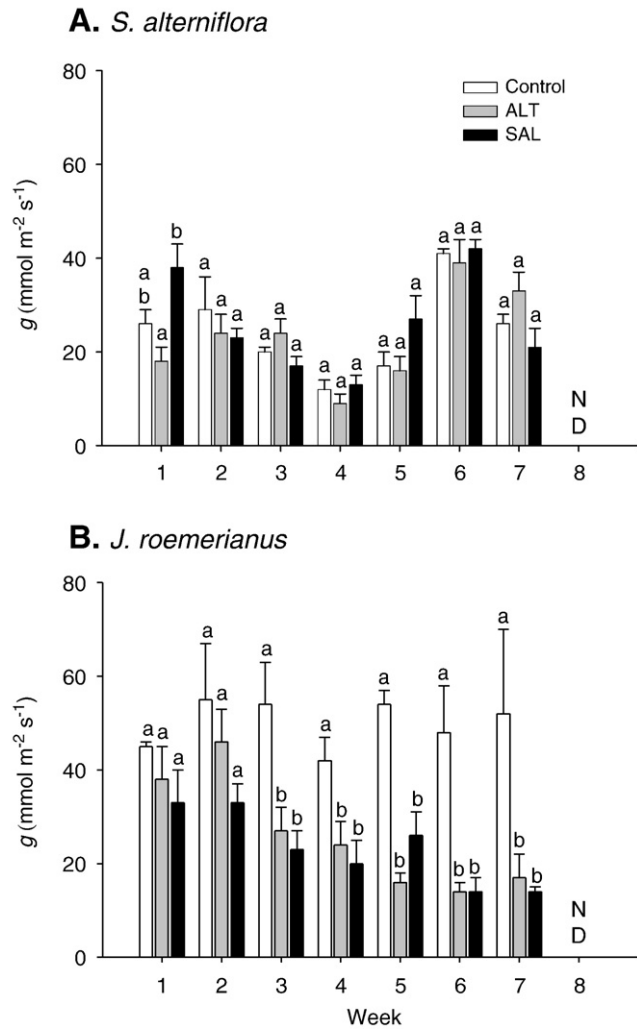


Fig. 4. Mean leaf conductance ($g; \text{mmol m}^{-2} \text{s}^{-1}$) for *S. alterniflora* (panel A) and *J. roemerianus* (panel B) receiving only freshwater (Control; white bars), alternating saltwater and freshwater weekly (ALT; seawater applied on even weeks), and only saltwater (SAL). Data are presented as means \pm 1 SE, and significant differences for each week are indicated by letters above the bars.

increase in bulk modulus elasticity (ϵ) for SAL treated plants, indicating a substantial increase in tissue rigidity after eight weeks of salt treatment ($p=0.018$; Table 3). Unlike the physiological modifications in pressure–volume relations observed in *S. alterniflora*, no significant changes in Ψ_{π} , P , or ϵ were observed in *J. roemerianus* ($p \geq 0.40$; Table 3).

Table 3

Plant water status parameters derived from pressure–volume analysis on *Spartina alterniflora* and *Juncus roemerianus* grown in 0 psu (Control), weekly alternations between 0 and 30 psu (ALT), or constant 30 psu (SAL).

Species (treatment)	Ψ_{π}^{sat} (MPa)	Ψ_{π}^{tlp} (MPa)	θ_{tlp} (%)	θ_{sym} (%)	P^{sat} (MPa)	ϵ (MPa)
<i>S. alterniflora</i>						
Control	$-1.16 \pm 0.08^{\text{a}}$	$-1.36 \pm 0.08^{\text{a}}$	$95.0 \pm 0.8^{\text{ab}}$	74.1 ± 2.0	$1.11 \pm 0.08^{\text{a}}$	$5.1 \pm 0.7^{\text{a}}$
ALT	$-1.39 \pm 0.15^{\text{ab}}$	$-2.40 \pm 0.27^{\text{b}}$	$90.9 \pm 1.2^{\text{a}}$	69.0 ± 4.8	$1.34 \pm 0.15^{\text{ab}}$	$10.4 \pm 1.7^{\text{ab}}$
SAL	$-1.82 \pm 0.04^{\text{b}}$	$-2.25 \pm 0.08^{\text{b}}$	$95.1 \pm 0.6^{\text{b}}$	68.9 ± 3.9	$1.76 \pm 0.05^{\text{b}}$	$18.0 \pm 5.3^{\text{b}}$
<i>J. roemerianus</i>						
Control	-0.68 ± 0.07	-0.92 ± 0.12	94.6 ± 1.3	79.2 ± 2.9	0.57 ± 0.08	10.4 ± 2.4
ALT	-0.54 ± 0.06	-0.90 ± 0.15	93.7 ± 1.5	81.4 ± 3.5	0.45 ± 0.05	16.6 ± 2.7
SAL	-0.55 ± 0.05	-0.75 ± 0.08	95.2 ± 0.8	83.2 ± 3.4	0.51 ± 0.07	15.7 ± 4.7

Parameters include osmotic potential at full saturation (Ψ_{π}^{sat}), osmotic potential at turgor loss point (Ψ_{π}^{tlp}), relative water content at turgor loss point (θ_{tlp}), symplastic water fraction (θ_{sym}), turgor pressure at saturation (P^{sat}), and bulk modulus of elasticity (ϵ). Statistical differences for each species are indicated by letters. Data are presented as means \pm 1SE.

4. Discussion

The observed physiological responses between ALT- and SAL-treatments were, for the most part, similar. However it is interesting to note that the ALT treatment responses to elevated salinity were frequently delayed, as reported for Ψ_{xylem} in both species, and for θ and Ψ_{leaf} in *J. roemerianus*. This suggests that the physiological adjustments, following pulsed-salinity stress, may extend beyond the stress event and, perhaps, allow these plants to endure future salinity pulses. This is consistent with observations on *S. alterniflora* where even low-level salt exposures, through weekly salt spray, produced extensive modifications in plant–water relations (Touchette et al., 2009).

In this study, *S. alterniflora* and *J. roemerianus* underwent substantial decreases in both Ψ_{leaf} and Ψ_{xylem} during salinity stress. However, the physiological processes involved in lowering tissue Ψ were mechanistically different between the two halophytes. In *S. alterniflora*, the lower tissue Ψ was primarily generated through an increase in tissue solute content (ions and/or organic osmolytes). The lower Ψ_{π}^{sat} and Ψ_{π}^{tlp} observed in these plants, coupled with no significant loss in tissue water content, indicates an active accumulation of osmotica following salt challenge. In contrast, while there were no significant changes in Ψ_{π}^{sat} and Ψ_{π}^{tlp} following salt treatments in *J. roemerianus*, tissue Ψ still decreased. We suspect that lower tissue water content in *J. roemerianus* during salt stress contributed greatly towards depressing tissue Ψ . Essentially, as tissue desiccation progresses, the existing solutes are confined into smaller volumes, effectively increasing osmolyte concentration without any increase in the number of solutes, thus lowering Ψ_{π} (Touchette and Frank, 2009). Under this scenario, the concentration of solutes is expected to return to initial levels following tissue rehydration. This is consistent with our observations where no significant differences were observed in Ψ_{π}^{sat} and Ψ_{π}^{tlp} between control and salt treated plants.

The apparent lack of any accumulation of osmotica in fully mature *J. roemerianus* during this investigation was unexpected and could be attributed to a sudden increase in salinity without acclimation. In a field study that considered spatial variations in plant–salinity relations of *J. roemerianus*, Ψ_{π}^{sat} and Ψ_{π}^{tlp} were clearly lower in plants exposed to the greatest salinities (Touchette, 2006). Furthermore, *J. roemerianus* has been shown to increase organic solutes (e.g., proline) when exposed to elevated salt (Cavaliere and Huang, 1979). We suspect that, in comparison to plants that are exposed to low salt levels during active periods of growth and development, mature fully-grown *J. roemerianus* acclimated only to freshwater, as in this study, may have a limited capacity to undergo osmotic adjustment following rapid and substantial increases in salinity. This is consistent with observations on other halophytes, in which older or non-acclimated plants had lower capacities to cope with elevated salinities, and that certain fixed modifications to salt may be determined by prevailing salinities at the time of tissue maturation (Hwang and Morris, 1994; Hester et al., 1998; Hester et al., 2001; Munns, 2002). Clearly, further

studies are necessary to determine when and under what conditions osmotic adjustments are made in this species.

Along with osmotic adjustment, changes in tissue elasticity can play an important role in plant–water relations during osmotic or water stress (Pardossi et al., 1998; Touchette, 2007). In this study *S. alterniflora* underwent a three-fold increase in tissue rigidity (as indicated by an increase in ϵ) when grown in high salinities. Other studies have also reported increased ϵ in plants experiencing elevated salts (Nabil and Coudret, 1993; Pardossi et al., 1998; Paliyavuth et al., 2004; Touchette, 2006), including a 30-fold increase in tissue rigidity when *Althernanthera philoxeroides* Mart. was grown in 400 mM solution of NaCl (Bolaños and Longstreth, 1984). It is possible that plants residing in aquatic environments, or other systems with an abundance of water, could benefit from more ridged tissues by allowing greater ease in transpirational water flux. That is, a small loss of water through transpiration can substantially decrease Ψ in ridged tissues, thereby allowing plants to exploit abundant water supplies to maintain high water throughput and a concomitant low Ψ (Bolaños and Longstreth, 1984; Touchette, 2006). Therefore a combination of sustained transpiration and increased tissue rigidity could produce lower tissue Ψ while minimizing overall tissue water depletions. Although we did not observe any significant changes in tissue elasticity in *J. roemerianus*, it is important to note that control *J. roemerianus* maintained considerably higher ϵ compared to *S. alterniflora*. Moreover, as indicated by the lower water content for salt treated *J. roemerianus*, any enhancement of water efflux could become problematic by promoting further desiccation.

No significant declines in leaf conductance (g) were observed in *S. alterniflora* during this study. Other investigations have reported diminished g due to high salinities in this plant, albeit inconsistent at times (Longstreth and Strain, 1977; Hwang and Morris, 1994). Hwang and Morris (1994), for example, observed striking declines in g when *S. alterniflora* underwent a step-wise increase in salinity from 5-, to 25-, to 40-ppt NaCl at five-day intervals. However, no significant reductions in g were noted when step-wise salinity decreased from 40-, to 25-, to 5-ppt NaCl. In contrast to *S. alterniflora* in this study, substantial reductions in g were observed in salt treated *J. roemerianus*. Decreased g is likely a more typical response in halophytes following exposure to salinities well above those necessary for optimal growth and development (Webb, 1966; Gorham, 1996; Koyro, 2006). Under such conditions, lowering g would minimize further water loss while allowing plants to respond to the prevailing stress and to reestablish favorable plant–water relations.

Based on the physiological modifications observed in this study, salt-tolerance appeared to be the principle response of *S. alterniflora* to salt stress. This includes a decrease in Ψ_{π} (suggesting osmotic adjustment), and an increase tissue rigidity (ϵ). In contrast, salt-avoidance was invoked by mature *J. roemerianus* through a decrease in g during stress. While both responses may be important in allowing the plant to survive short periods (weeks to months) of abrupt salinity stress, salt-avoidance would seemingly compromise the plant's long-term survival as decreased g would also limit carbon uptake/fixation and plant productivity (Morgan, 1984).

Interestingly, unlike drought responses which typically involve some combination of tolerance and avoidance, both halophytic species in this study responded with only one of the two processes. It is possible that additional experiments that alter the duration and degree of salt challenges (perhaps higher salinities for *S. alterniflora* and lower salinities for *J. roemerianus*) may eventually induce some combination of salt-tolerance and salt-avoidance in these species. This would be consistent with other studies that have demonstrated salt-tolerance in *J. roemerianus* (Cavaliere and Huang, 1979; Touchette, 2006) and salt-avoidance in *S. alterniflora* (Longstreth and Strain, 1977; Hwang and Morris, 1994).

In conclusion, the physiological mechanisms employed during salt stress by halophytes are analogous to drought-stress responses

observed in many terrestrial plant species. Although water-stress processes have been well characterized in economically important terrestrial plants, this is one of the first studies that demonstrate dissimilar physiological responses in two different coastal-marsh macrophytes receiving similar salt treatments. While the responses to high salinity in *S. alterniflora* involved salt-tolerance and thereby allow the plants to reside in prevailing conditions indefinitely, the response by *J. roemerianus* was primarily salt-avoidance which, in the absence of any further physiological adjustment, would likely limit the amount of time a plant could survive under persistent stress conditions. These physiological responses are consistent with observed zonation patterns in these plants, wherein *S. alterniflora* resides in the lower marsh and must contend with long-term salt exposure. In contrast, *J. roemerianus* often inhabits the upper reaches of salt- and estuarine-marshes where salinities are considerably lower and where salt stress often involves episodic exposures to elevated salt levels.

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