Plant Suitability for Floating Treatment Wetland Applications in Brackish Waters

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Abstract

Brackish water bodies provide critical ecosystem services supporting human and environmental health in coastal regions. Mitigation of contaminants in brackish waters is critical. Floating treatment wetlands (FTWs) are a remediation technology typically applied to improve water quality in freshwater systems. However, the applicability of FTWs to waters with fluctuating salinity has not been determined. The goal of this study was to quantify the growth and survival of four plant species and determine which aquatic macrophytes are suited for use in FTWs deployed in brackish waters. This study trialed four species common to salt marshes of the Southeastern USA (*Distichlis spicata, Juncus roemerianus, Spartina alterniflora,* and *Spartina patens*) grown under three salinity exposures (0.5, 5.0, and 18 $g \cdot L^{-1}$) over 7 weeks. We also quantified macro- and micro-nutrient concentrations in these species to enable determination of how plant growth in FTWs may alter nutrient partitioning in roots and shoots. The ratios of nutrients fixed in tissues of plants grown in the salt marsh vs. those grown in FTWs shifted. The ratio of Na:Ca within tissues were higher for plants grown in the saltmarsh than those grown in the FTWs. Regarding biomass production, *D. spicata* and *J. roemerianus* grew very little, regardless of salinity exposure. *J. roemerianus* did not survive at 18 $g \cdot L^{-1}$ salinity. *S. alterniflora* and *S. patens* had the highest biomass production and final nutrient concentrations in tissues across all salinities. These trials indicated that of the four species tested, *S. alterniflora* and *S. patens* hold the most promise for FTW application in brackish settings.

Keywords: salt tolerance; halophytes; phytoremediation; stormwater pollution

1. Introduction

Nutrient pollution of coastal waters has increased in the past decades due to anthropogenic activities and represents a threat to the ecological and economic sustainability of coastal and marine environments worldwide. Coastal ecosystems provide ecological services of great importance: shoreline buffering, storage and cycling of nutrients, conservation of biodiversity, and maintenance of water quality (Burke et al. 2001). Amongst coastal ecosystems, brackish water ecosystems (e.g., wetlands, salt marshes, estuaries, mangroves, and coastal lagoons) are most affected by anthropogenic activities (Torres-Alvarado et al. 2019). Brackish water ecosystems represent the interface between terrestrial and marine environments and are influenced by the combination of seawater and freshwater and a series of biological and ecological features fundamental to the sustainability of all coastal ecosystems (Torres-Alvarado et al. 2019). Major contaminants contributing to aquatic pollution in these systems include persistent organic chemicals, nutrients, oils, heavy metals, pathogens, sediment, litter, and debris (Williams 1996).

Floating treatment wetlands (FTWs) are a remediation technology that both remove aquatic contaminants (e.g., nutrients, metals, pathogens, and oil) and provide habitat while being economically feasible to install in existing water bodies (Colares et al. 2020; Garcia Chance et al. 2022). Floating treatment wetlands consists of emergent vegetation that grows hydroponically on structures (hereafter – FTW scaffolds) floating on the waters' surface (Headley and Tanner 2008). This nature-based technology has been demonstrated to diminish contaminants present in wastewater from various industries, agricultural irrigation return flow, stormwater runoff, municipal sewage, and oil-contaminated waters (Afzal et al. 2019; Colares et al. 2020; White and Cousins 2013). However, little research has been conducted on their applicability in brackish waters (Sanicola et al. 2019; Lyu et al. 2020).

The salinity of brackish waters has been parsed into three categories: oligohaline (0.5 -5.0 parts per thousand or $g \cdot L^{-1}$), mesohaline (5.0- 18.0 $g \cdot L^{-1}$), and polyhaline (18.0 to 30.0 $g \cdot L^{-1}$) (Montagna et al. 2012). High salinity impacts plant metabolism and causes stress. Salinity stressed plants often have smaller leaves, experience stomatal closure, decreased photosynthesis, loss of turgor pressure due to water loss from osmotic imbalance, and necrosis (Rahnama et al. 2010; James et al. 2011). Some plant species tolerate salinity stress (Zhang and Shi 2013).

Halophytes are defined as plant species that can survive and reproduce in environments with salt concentrations equal or higher than 200 mM NaCl (12 $g \cdot L^{-1}$) (Flowers and Colmer 2008). Typically, salinity tolerance is defined within soil-based systems, yet the salinity tolerance of plants grown in hydroponic systems tends to differ (Grattan and Grieve 1998). The ratio of cations within tissues and availability in solution likely have stronger influence on salinity tolerance in hydroponic systems than soil-based systems where the mineral strata contribute salts that enhance salinity tolerance (Grattan and Grieve 1998).

Characteristics to consider when selecting plant species used in FTWs include root system density, availability (local or regional), nutrient storage potential (i.e., biomass), and adaptability to site-specific water chemistry and climatic conditions (Garcia Chance et al. 2020; Shahid et al. 2018). Additionally, for applicability of FTW in brackish waters, plant species must tolerate salinity. Few published studies have focused on the application of FTWs in brackish waters for the removal of contaminants, specifically nutrients (Karstens et al. 2021). No studies, to our knowledge, have studied the application of FTWs in brackish stormwater ponds. Therefore, the goal of this study was to quantify the growth and survival of four plant species and to determine which aquatic macrophytes are suited for use in FTWs deployed in brackish pond systems. These results will begin to inform future salt-tolerant plant selections for FTW applications in estuarine stormwater ponds.

2. Materials and methods

2.1. Experimental design

Experiments were designed as a 3x4 factorial with treatments including salinity (0.5, 5.0, and 18 $g \cdot L^{-1}$) and plant species (*Distichlis spicata* (L.) Greene (saltgrass), *Juncus roemerianus* Scheele (needlegrass rush), *Spartina alterniflora* Loisel. (smooth cordgrass), and *Spartina patens* (Aiton) Muhl. (saltmeadow cordgrass). The three salinity concentrations were selected based on the lowest concentration of each salinity category established for brackish waters [oligohaline (0.5 - 5.0 $g \cdot L^{-1}$), mesohaline (5.0- 18.0 $g \cdot L^{-1}$), and polyhaline (18.0 to 30.0 $g \cdot L^{-1}$)] to provide exposures across the range of brackish waters (Montagna et al. 2012). Each treatment had ten plant experimental units.

The salinity tolerance of four salt marsh macrophytes was evaluated over seven weeks of 2021 summer (June – July) at the Baruch Marine Field Laboratory in Georgetown, SC. Experimental

Figure 1. Experimental set up of 296 L tanks (*n* =12, left) with floating treatment wetlands installed, and the mixing tanks (*n* = 2, right) for dilution of brackish water pumped from the adjacent estuary to attain the three salinity levels (0.5, 5.0, and 18 $g \cdot L^{-1}$).

units consisted of 12 tanks (0.97 m in width, 0.40 m in height) with a total capacity of 296 L per tank (Figure 1). Each tank was filled with 185 L of saltwater diluted to the target concentration using unchlorinated well-water (0.5 $g \cdot L^{-1}$). The 0.5 $g \cdot L^{-1}$ treatment used unadulterated well water.

On June 8, 2021, *D. spicata*, *J. roemerianus*, *S. alterniflora*, and *S. patens* were collected from the saltmarsh at North Inlet–Winyah Bay Estuary, Georgetown, SC. Plant collection was permitted by the landowner, the Belle W. Baruch Foundation, which manages 16,000 acres of upland and wetland habitat reserved for research and education. Co-author William Strosnider identified each population. Specimen vouchers collected from the populations were deposited at the A.C. Moore Herbarium at the University of South Carolina (USCH) with accession numbers 128771 (*S. alterniflora*), 128772 (*S. patens*), 128773 (*D. spicata*), 128774 (*J. roemerianus*).

Plants were collected locally to ensure genotypes adapted to site-specific water quality and climactic conditions. Plants selected for harvest were similar in size. The harvest was conducted with minimal disturbance to the ecosystem and other ongoing research projects. Each plant replicate was separated from the harvested plant mass based on morphological characteristics: 3 × 3 cm clump for *S. patens*, 8-shoot clumps for *D. spicata* and *J. roemerianus*, and individual shoots for *S. alterniflora* (Figure 2). Sediment was gently washed off the roots, then shoot height (cm), and fresh weight (g) of each plant replicate were recorded. Plants were placed in aerator cups and inserted into 65 × 55 cm, 1 cm thick, floating mats (Beemats, New Smyrna Beach, FL).

Figure 2. Plant were sorted into similar size groupings according to plant growth variability of *Distichlis spicata*, *Juncus roemerianus*, *Spartina alterniflora*, and *Spartina patens* (left to right).

FTWs were installed on batch water system tanks filled brackish water on June 8, 2021 (Day 1). Thin plastic rings were placed on the aerator cups around the plant shoots, to help plants remain upright for the initial two weeks of the experiment (Supplemental Figure 1). At the beginning of week 3, the plastic rings were removed, and coir fiber mats were cut to size (7.5 × 10.5 cm) and placed around the plants in the aerator cups, providing support.

2.2. Brackish water dilutions

Brackish waters for the trials were prepared by diluting saltwater to target salinity concentrations of 0.5, 5.0, and 18 $g \cdot L^{-1}$ using non-chlorinated, fresh well water. Table 1 presents the average physicochemical characteristics of fresh and brackish waters used for the experiments. Dilutions were prepared in two mixing tanks (2.5 m wide, 0.9 m high, with a volume of 4,418 L) by mixing seawater pumped directly from the salt marsh with fresh well water (Figure 1). Salinity of the water in the experimental tanks was maintained consistently at the target salinity concentrations, with negligible influence of evaporation or rainfall, as water in the experimental tanks was replaced weekly. Because the experimental site was directly adjacent to the Winyah Bay National Estuarine Research Reserve, we chose not to add additional nutrients to any solutions to minimize the potential for nutrient escape into the research reserve. Rather we chose to evaluate a "worst-case scenario" where plants would be exposed to salinity without the benefit of additional nutrients or a lengthier acclimatization period to help mitigate the stress, as in many FTW applications plants are not afforded ideal planting conditions by vendors and clients.

Table 1. Mean water quality of waters used for preparation of brackish water treatments used in the study.

	Freshwater	Seawater
pH	8.60 (0.00) [z]	7.37 (0.08)
Salinity ($g \cdot L^{-1}$)	0.50 (0.00)	31.8 (0.91)
DO ($mg \cdot L^{-1}$)	2.18 (0.08)	2.88 (0.32)
TN ($mg \cdot L^{-1}$)	-	0.089 (0.00)
PO_4-P ($mg \cdot L^{-1}$)	-	0.017 (0.00)
Cl ($mg \cdot L^{-1}$)	55.5 (7.16)	20058[y]
Na ($mg \cdot L^{-1}$)	234 (3.60)	11174
S ($mg \cdot L^{-1}$)	1.34 (0.04)	2811
Mg ($mg \cdot L^{-1}$)	0.12 (0.06)	1331
Ca ($mg \cdot L^{-1}$)	0.82 (0.21)	427
K ($mg \cdot L^{-1}$)	4.97 (0.15)	414
B ($mg \cdot L^{-1}$)	1.04 (0.01)	6.01
P ($mg \cdot L^{-1}$)	<0.020	0.071
Zn ($mg \cdot L^{-1}$)	<0.010	0.0004
Cu ($mg \cdot L^{-1}$)	< 0.010	0.00019
Fe ($mg \cdot L^{-1}$)	<0.010	0.00003
Mn ($mg \cdot L^{-1}$)	< 0.010	0.00002

[z] Mean values (standard error) of the mean.
[y] Values for seawater macro and micronutrients were not measured during the experiment. Thus, we used global seawater means from Millero (2014) and Bruland et al. (2014).

One day before experiment initiation, the three salinities were prepared in the tanks using a total volume of 1,136 L per dilution and distributed to the experimental tanks using a submersible pump (91570 1/2 HP; Superior Pump, Minneapolis, MN) connected to a hose and pipe distribution system. Brackish water in the experimental tanks was prepared and changed weekly. Nutrient analyses data of the seawater was provided by the North Inlet – Winyah Bay National Estuarine Research Reserve. The fresh well water samples were collected at weeks 3, 5, and 7 (n=3) and analyzed for pH, electrical conductivity, and nutrient elements using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis.

2.3. Water and plant health data

For each tank (n=12), water pH, DO ($mg \cdot L^{-1}$), specific conductivity (µS/cm), salinity ($g \cdot L^{-1}$), and water temperature (°C) were measured weekly using a regularly calibrated multiparameter hand-held sonde (YSI ProDSS 626870-2; YSI Inc., Yellow Springs, OH). Plant height, survival

(yes/no), colorimetric rating (1 to 5), and visual health rating (1 to 5) were recorded every seven days. Colorimetric rating was performed by developing a scale based on the intensity of green and presence of chlorosis (Supplemental Figure 2), with a 5 representing plants with consistent green coloration, a 3 representing plants that are mainly chlorotic, and a 1 representing completely necrotic plants.

Visual health rating accounted for stress symptoms such as leaf browning, leaf wilting, leaf tip burning, and leaf rolling (Supplemental Figure 3). For visual health scale, plants with no stress symptoms were classified with a rating of 5, plants presenting one symptom were a 4, plants with two or three symptoms were a rating of 3, plants with four to five signs were considered a 2, and plants with more than five symptoms were considered a 1. The number of new shoots was recorded weekly, starting at week 3 of the experiment. At day 0, five representative plants per species (n=5) were sacrificed to determine initial fresh and dry mass and nutrient content. The plant roots and shoots were separated, dried at 60 °C for five days, and dry weight recorded.

2.4. Plant tissue samples

All plant shoots (above-mat biomass) and roots (below-mat biomass) (n=10, per treatment) were harvested on July 27, 2021, at the conclusion of the trial. After harvest, fresh weights (g) of roots and shoots were recorded. The shoots and roots were dried at 60 °C until constant mass and the dry weight were recorded. Net plant biomass was calculated by subtracting the initial weight from the biomass weight at harvest. The root:shoot ratio was calculated by dividing the dry mass of roots by the dry mass of shoots.

Three plants per species were used for determination of initial tissue nutrient content. These plants were from the same batch harvested from the marsh and used to plant the FTWs. Five plants per treatment (n=5) were used for the determination of tissue nutrient content. The roots and shoots were ground using a Wiley® mini-mill (Thomas Scientific, Swedesboro, NJ) with a 0.6 mm mesh size. Plant tissues were digested by microwave assisted nitric acid digestion prior to ICP-OES (iCAP 6500, Thermo Scientific, Waltham, MA) analysis at the Clemson University Agricultural Services Laboratory. Following digestion, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), sulfur (S), and sodium (Na) concentrations in plant tissues were determined via ICP-OES with calibration standards confirmed in the middle and end of each analytical run to ensure reliable sample

analysis. Ratios of nutrients fixed in plant tissues were calculated using $mg \cdot g^{-1}$ of Ca, Mg, K, and Na fixed in plant tissues.

2.5. Data analysis

Effects of treatments were assessed using Analysis of Variance (ANOVA) and Least Squares Effect Testing. When treatment effects were significant (p or $f < 0.05$), post-hoc means comparisons were conducted using the Tukey HSD option of LSMeans. Normality assumptions were evaluated using residuals, if data were non-normal and non-transformable, the Kruskal-Wallis test (non-parametric alternative to the One Way ANOVA) was used to determine treatment differences. All statistical analyses were conducted using JMP Pro 16 (SAS Institute Inc., Cary, NC). All data are reported as mean ± standard error unless otherwise noted.

3. Results and discussion

3.1. Water quality parameters

Physicochemical parameters of water in the experimental tanks were similar throughout the duration of the experiment across plant species (Figure 3). Water pH differed across salinity treatments ($p < 0.0001$) and averaged 8.7 ± 0.02 in the 0.5 $g \cdot L^{-1}$ salinity, 8.2 ± 0.02 in the 5 $g \cdot L^{-1}$

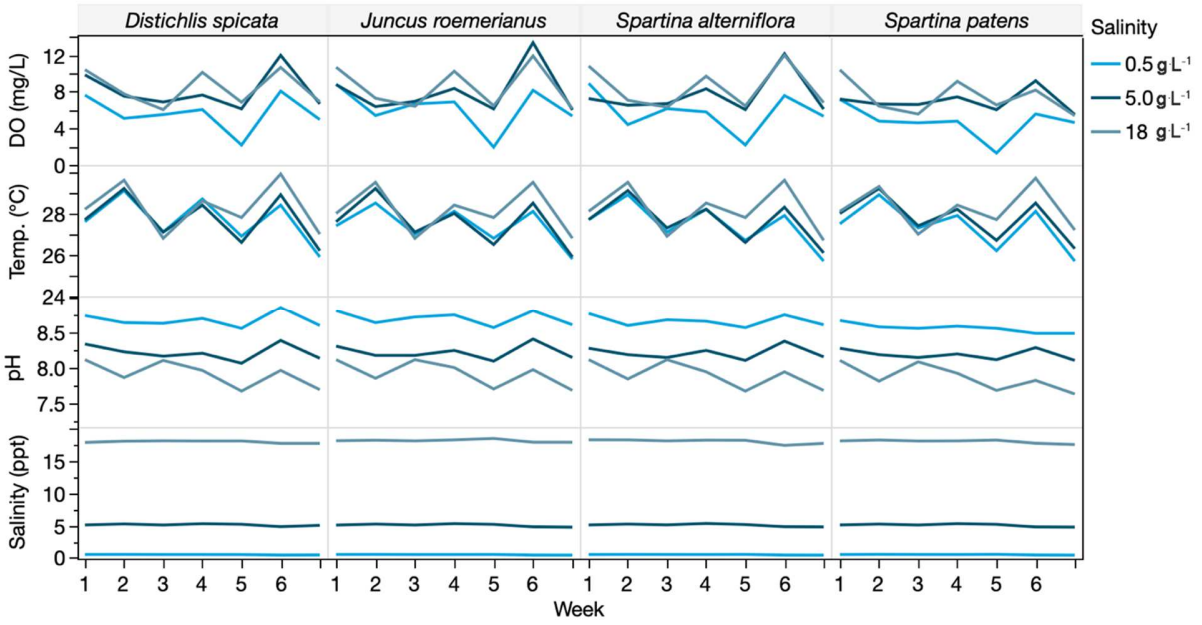

Figure 3. pH, salinity ($g \cdot L^{-1}$), temperature, and dissolved oxygen (DO) at each of the seven sampling events in the experimental tanks throughout the duration of the experiment.

salinity, and 7.9 ± 0.03 in the 18 g·L^{-1} salinity. Water temperature was not statistically different across all salinities and treatments. For all plant species, DO increased with increased salinity (p < 0.0001), perhaps due to differences in phytoplankton communities. Dissolved oxygen concentrations (mg·L^{-1}) were 5.6 ± 0.4, 7.7 ± 0.4, and 8.2 ± 0.4, for the 0.5, 5.0, and 18 g·L^{-1} salinity treatments, respectively. Dissolved oxygen concentrations were similar, regardless of plant species.

3.2. Plant performance

All *D. spicata*, *S. alterniflora*, and *S. patens* plants survived, regardless of salinity treatment. At 0.5 g·L^{-1} salinity, 90% of *J. roemerianus* plants survived. At 5.0 g·L^{-1} salinity 80% survived, and at 18 g·L^{-1}, no *J. roemerianus* plants survived. At the experiment's conclusion, all indicators of plant health (colorimetric rating and visual health index) for *D. spicata*, *J. roemerianus*, and *S. patens* decreased with higher salinity (p <0.0001). Plants exposed to 18 g·L^{-1} salinity presented more stress symptoms including leaf firing and wilting compared to symptoms observed at the 0.5 g·L^{-1} salinity. Contrarily, *S. alterniflora* colorimetric ratings (p < 0.0534) and visual health ratings (p < 0.3415) were similar across all salinity treatments, with fewer stress symptoms (i.e., leaf burning, and leaf wilting) evident in contrast to the other plant species evaluated.

Production of new shoots decreased with higher salinity concentration for *D. spicata*, *J. roemerianus*, and *S. patens* (p <0.0001). *D. spicata* grown at 0.5 g·L^{-1} salinity had 8.6 ± 1.1 new shoots, 6.0 ± 1.2 new shoots at 5.0 g·L^{-1}, and 4.0 ± 0.8 new shoots at 18 g·L^{-1} salinity. *Juncus roemerianus* averaged 2.6 ± 1.3 new shoots when grown at 0.5 g·L^{-1} salinity, 0.3 ± 0.2 new shoots at 5.0 g·L^{-1} salinity, and growth of no new shoot at 18 g·L^{-1} salinity. *Spartina patens* had 14.8 ± 1.6, 9.0 ± 1.7, and 6.7 ± 1.5 new shoots at salinity concentrations of 0.5, 5.0 g·L^{-1}, and 18 g·L^{-1}, respectively. Regardless of salinity, *S. alterniflora* produced a similar number of new shoots over the experiment (1.8 ± 0.3).

Final plant biomass production varied by plant species (p < 0.0001) and salinity level (p < 0.0423). *Distichlis spicata* and *J. roemerianus* lost biomass over the seven weeks of the experiment, while *S. alterniflora* and *S. patens* gained biomass (Figure 4). Increasing salinity concentration (0.5 ⇒ 5.0 ⇒ 18 g·L^{-1}), resulted in incremental increases in biomass losses by *D. spicata* -0.8 g ± 0.5 ⇒ -1.7 g ± 0.4 ⇒ -2.4 g ± 0.4, respectively (p < 0.0342). Aschenbach (2006)

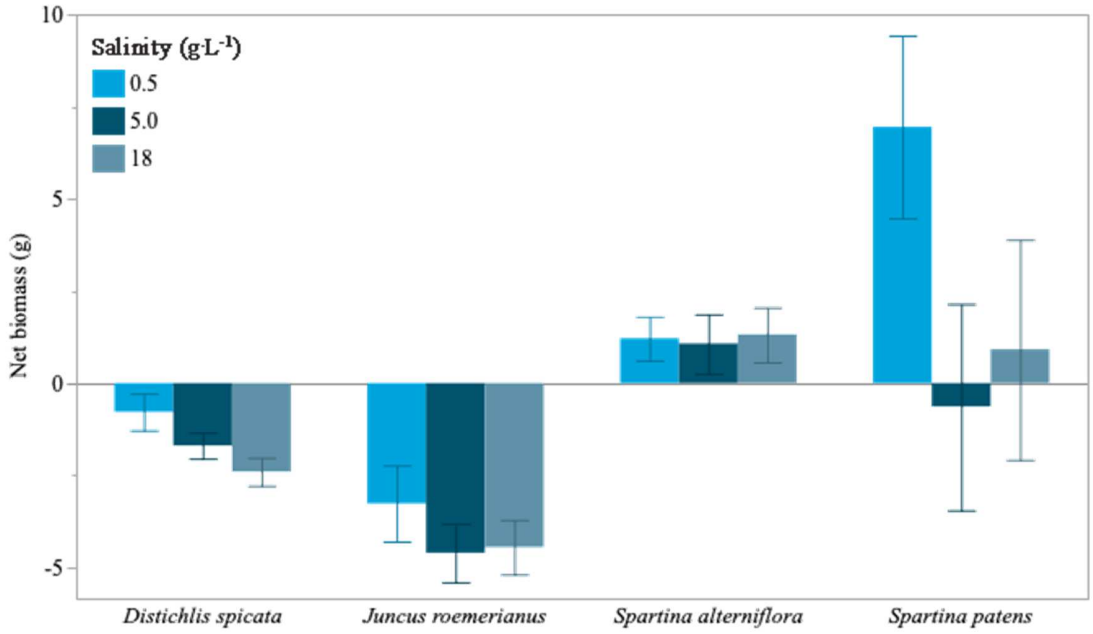

Figure 4. Net dry plant biomass at the end of the experiment normalized by subtracting the initial weight from the final weight.

also noted the growth of *D. spicata* in sand culture was negatively affected at salinity concentrations above 7.9 g·L^{-1}. *Juncus roemerianus* had the least net change in biomass at the 0.5 g·L^{-1} salinity treatment (-3.3 g ± 1.0), and similar biomass losses in 5.0 g·L^{-1} (-4.6 g ± 0.8) and 18 g·L^{-1} (-4.4 g ± 0.7) salinity levels.

Biomass production across all salinities was similar for *S. alterniflora*. Vasquez et al. (2006) also reported that *S. alterniflora* developed new shoots and increased biomass across salinities

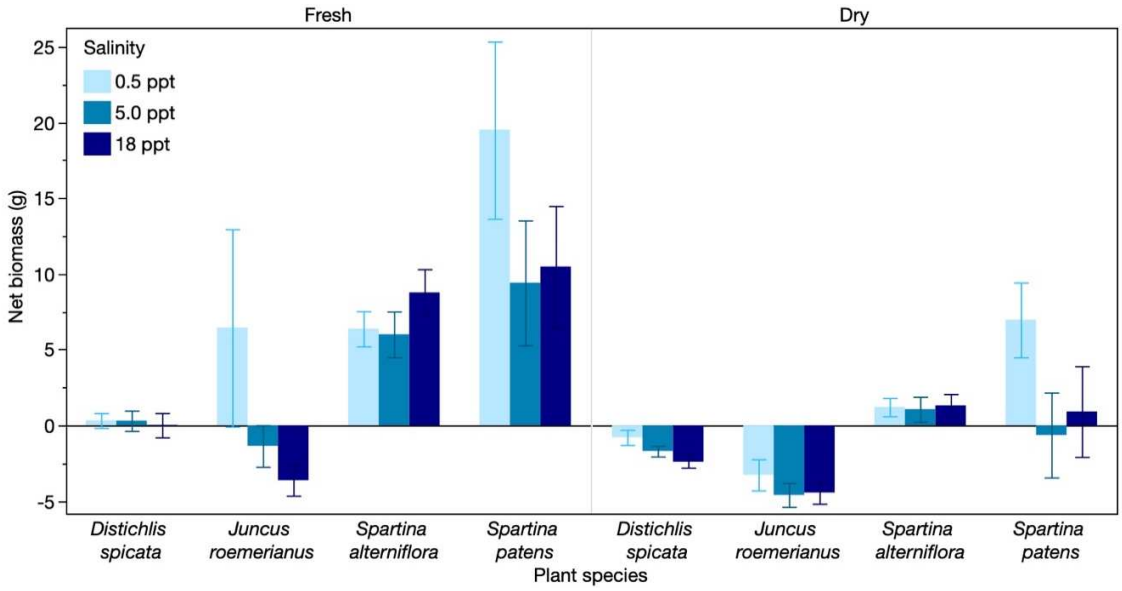

Figure 5. Net dry and fresh plant biomass at the end of the experiment after exposure to ascending concentrations of salinity (g/kg) normalized by subtracting the initial weight from the final weight.

225 ranging from 0.57 to 34 $g \cdot L^{-1}$ in soil-based greenhouse experiments. Biomass accumulation by *S.*
226 *patens* was highest at 0.5 $g \cdot L^{-1}$ salinity (7.0 g ± 2.5), though biomass accumulation at 0.5 $g \cdot L^{-1}$
227 did not differ from that at 5.0 (-0.64 g ± 2.8) and 18 $g \cdot L^{-1}$ (0.92 g ± 3.0) salinities ($p < 0.0539$),
228 due to high variability within treatments. Similarly, in a study performed by Ewing et al. (1997),
229 *S. patens* biomass production did not decrease at salinities from 0 to 7.0 $g \cdot L^{-1}$, but biomass
230 decreased, and stress signs were noted at salinities of 14 $g \cdot L^{-1}$ and higher in the saturated soils of
231 a Louisiana marsh.

232 The low survival rate and low biomass production of *J. roemerianus* may have been a result of
233 an age-specific response of the plant material used (mature plants) combined with salinity stress,
234 transplant shock, and lack of adequate acclimation period (Touchette et al. 2012). In some
235 halophytes, older plants or non-acclimated plants have more difficulty growing under newly
236 introduced, high salinity conditions because salt-tolerance adaptations are most likely to occur
237 with growing tissues (Hwang and Morris 1994; Hester et al. 1998; Hester et al. 2001; Munns
238 2002). *Juncus roemerianus* tolerated salinities higher than 40 $g \cdot L^{-1}$ in saturated soils (Stalter and
239 Lonard 2023; Eleuterius 1989; Christian et al. 1990; Brinson and Christian 1999; Pennings et al.
240 2005), which contrasts our findings in which this plant species did not survive at 18 $g \cdot L^{-1}$,
241 indicating factors other than salinity likely influenced its survival. *Distichlis spicata* also showed
242 the slowest adaptation after transplant, most likely because of the slow growth rate of this plant
243 (Aschenbach 2006). *Spartina alterniflora* is a lower marsh plant that tolerates high salinity levels
244 via physiological mechanisms such as osmotic adjustment and increased tissue rigidity
245 (Touchette et al. 2009). *Spartina alterniflora* and *S. patens* accumulate proline and glycine
246 betaine, organic solutes that aid in osmotic adjustment, which may have supported consistent
247 growth of the plants across salinity treatments (Cavalieri and Huang 1981; Hester et al. 2001).

248 *3.3. Initial nutrient status of plants*

249 The root:shoot ratio of plants harvested from the saltmarsh were similar ($p = 0.554$; Table 2).
250 The root:shoot ratio of *S. alterniflora* harvested for this experiment (0.73) was similar to the
251 values reported by Tang et al. (2022) which ranged from 0.79 to 0.85. However, the value
252 obtained in our study differed dramatically from the value reported by Ornes and Kaplan (1989),
253 which was 5.2. This likely is an artifact of plant collection technique, with the Ornes and Kaplan
254 (1989) root:shoot ratio calculated from harvested shoot tissues and estimates of root biomass

made from soil cores; whereas our estimates were made after completely removing the plants from the saltmarsh. Regardless, this root:shoot data disparity may indicate potential for substantial investment in root tissue biomass which indicates potential for nutrient partitioning within root tissues in FTWs.

Of all elements tested, Na and K were present at the highest concentration in all plants (Table 2, Supplemental Table 1). *Spartina alterniflora* consistently had the highest concentration of all elements present, save for Cu, which was higher in *D. spicata* and *J. roemerianus* ($p < 0.0001$). Ornes and Kaplan (1989) reported P (1481 mg·kg^{-1}), K (7201 mg·kg^{-1}), Ca (1342 mg·kg^{-1}), Mg (3248 mg·kg^{-1}), and S (7981 mg·kg^{-1}) values for *S. alterniflora*, harvested in the same saltmarsh where plants were harvested for this experiment. The reported concentrations were similar for most elements, differences could be reflective of the season in which the tissues were collected or the relative maturity of the plant tissues (Kilcher 1981). DeLaune and Pezeshki (1988) also reported macro- and micro-nutrient concentrations in tissues of *S. alterniflora* harvested near Leeville, Louisiana (creekside and inland locations) and average values reported were similar for P, Mn, and Cu, substantially higher (K: 11790 mg·kg^{-1}; Na: 48207 mg·kg^{-1}; Ca: 10578 mg·kg^{-1}) or lower (Fe: 155 mg·kg^{-1}; Mg: 2448 mg·kg^{-1}; Zn 12.8 mg·kg^{-1}).

Table 2. Mean (standard error) of the *initial* dry weight, root:shoot ratio (dry mass root ÷ dry mass of shoots), and nutrient concentrations in whole plants (n =5) harvested from the saltmarsh at the North Inlet–Winyah Bay Estuary, Georgetown, SC.

	Distichlis spicata	***Juncus roemerianus***	***Spartina alterniflora***	***Spartina patens***
Dry weight (g)	5.00 (0.42)	11.5 (3.17)	4.87 (0.78)	17.9 (1.78)
Root:shoot	0.80 (0.13)	0.84 (0.13)	0.73 (0.14)	0.61 (0.07)
P (mg·kg^{-1}) [z]	692 (43.3) [b]	1000 (83.7) [a]	1100 (28.4) [a]	702 (36.3) [b]
K (mg·kg^{-1})	5440 (315) [b]	7590 (328) [a]	8450 (277) [a]	5680 (244) [b]
Ca (mg·kg^{-1})	1200 (75.6) [c]	1240 (113) [c]	2810 (198) [a]	1780 (59.9) [b]
Mg (mg·kg^{-1})	1740 (43.8) [b]	1750 (48.1) [b]	4920 (90.9) [a]	1100 (87.7) [c]
Zn (mg·kg^{-1})	21.2 (1.86) [b]	31.9 (4.91) [b]	50.2 (3.59) [a]	26.5 (0.96) [b]
Cu (mg·kg^{-1})	6.73 (0.64) [a]	6.71 (0.32) [a]	4.76 (0.10) [b]	4.38 (0.23) [b]
Mn (mg·kg^{-1})	52.3 (4.29) [ab]	52.7 (6.32) [ab]	73.2 (7.36) [a]	45.6 (2.43) [b]
Fe (mg·kg^{-1})	764 (155) [ab]	432 (56.3) [b]	1363 (214) [a]	505 (191) [b]
S (mg·kg^{-1})	2310 (34.0) [b]	2080 (22.4) [c]	4190 (91.4) [a]	2220 (41.5) [bc]

Na (mg·kg^{-1})	7900 (320) [b]	8580 (786) [b]	27600 (735) [a]	7660 (231) [b]

[z] Least squares analyses of variance (ANOVA), means within rows were separated using Tukey HSD (p < 0.05). Means followed by the same letter do not differ.

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276 *3.4. Final nutrient status of plants*

277 Nutrients absorbed by the plants harvested from the FTW varied by plant species (p < 0.0001)
278 and salinity concentration (p < 0.0001) (Tables 3 and 4, Supplemental Figures 4 -13). The root to
279 shoot ratio of the harvested plants was similar, regardless of species or salinity treatment (p =
280 0.0846). Of all plants evaluated, *S. alterniflora* typically contained the highest total mass of
281 macronutrients [Mg (p <0.0001), S (p < 0.0001), Na (p = 0.0005)] and micronutrients [Mn (p =
282 0.0009), Zn (p = 0.0042)] when grown in 18 g·L^{-1} salinity, save for P (p = 0.2686), K (p =
283 0.1616), Ca (p = 0.1154), Fe (p = 0.0712), and Cu (p =0.4990), which were similar across
284 salinity treatments. Nutrient concentrations in *S. patens* were also highest at the 18 g·L^{-1} salinity
285 (p = 0.0130) for most nutrients save K (p < 0.0001), Zn (p < 0.0001), and Cu (p = 0.0036), which
286 were highest in *S. patens* grown in the 0.5 g·L^{-1} salinity treatment. In a study comparing
287 elemental concentrations in leaf tissue in *S. alterniflora* and *S. patens*, Tobias et al. (2014)
288 reported that Ca, Mg, Mn, N, P, K, and Zn concentrations were higher in *S. alterniflora*, while
289 Al, B, Cu, Fe, S, and Na were similar in both species (Tobias et al. 2014).

290 Additionally, Tobias et al. (2014) also noted that Na concentrations in leaf tissue of *S.*
291 *alterniflora* were highest when salinity ranged from 15 to 20 g·L^{-1}; positing that *S. alterniflora*
292 has more ion selectivity than *S. patens*, as *S. alterniflora* absorbs more K than *S. patens* when
293 grown under the same conditions (Hester et al. 2001; Tobias et al. 2014). Final nutrient
294 concentrations in *Distichlis spicata* were also consistently highest at 18 g·L^{-1} salinity treatments
295 for most elements, save K (p = 0.4875), where salinity treatment did not influence the
296 concentration of K in tissues. Final nutrient concentrations in *Juncus roemerianus* were greater
297 in 18 g·L^{-1} salinity exposures for all elements save for P and Cu (p = 0.9899 and 0.6927,
298 respectively), where all treatments had similar concentrations; K (p = 0.0005), where 0.5 and 5.0
299 g·L^{-1} salinity treatments were higher; and Zn (p = 0.0041) which was highest in plants grown at
300 0.5 g·L^{-1} salinity.

301 Higher nutrient concentrations in plants grown in the 18 g·L^{-1} salinity treatment would also
302 certainly be influenced by the seawater containing far more salts than the freshwater used for the

303 0.5 g·L^{-1} salinity treatments. Generally, root tissues contained more P, K, Mg, Fe, S, Na, Cu, Mn,
304 and Zn than did plant shoots, regardless of plant species ($p < 0.0001$, Supplemental Tables 2 and
305 3). Though species did sometimes alter these partitioning trends, for instance, P final
306 concentration in plant tissues was the highest in the roots of *S. patens*, *J. roemerianus*, and *D.*
307 *spicata*, regardless of salinity treatment ($p < 0.0001$) but higher in the shoots of *S. alterniflora* (p
308 = 0.0185; Supplemental Table 2). Similar trends were true for K, which was highest in the roots
309 of all plants save *J. roemerianus*.

310 **Table 3.** Mean (standard error) root:shoot ratio and macronutrient concentrations in plants harvested at the conclusion of the 7-week
311 experiment.

Plant species	Salinity ($g \cdot L^{-1}$)	Root: Shoot	P ($mg \cdot kg^{-1}$)		K ($mg \cdot kg^{-1}$)		Ca ($mg \cdot kg^{-1}$)		Mg ($mg \cdot kg^{-1}$)		S ($mg \cdot kg^{-1}$)		Na ($mg \cdot kg^{-1}$)	
Distichlis spicata	0.5	1.09 (0.32)	559 (36.5)	b	4110 (290)	cd	1530 (44.0)	a	896 (35.1)	c	1130 (47.8)	b	2800 (117)	c
	5.0	0.97 (0.13)	532 (19.7)		4190 (114)	cd	2860 (211)		2170 (58.2)		1540 (27.0)		3980 (292)	
	18	1.15 (0.16)	698 (21.0)		4500 (258)	c	3910 (445)		2560 (103)		2390 (111)		6170 (264)	
Juncus roemerianus	0.5	0.89 (0.14)	806 (75.1)	a	5560 (426)	bc	1720 (42.0)	b	1560 (103)	b	1580 (45.7)	b	4730 (306)	b
	5.0	0.65 (0.11)	818 (57.4)		4200 (457)	cd	1780 (89.4)		2720 (78.1)		1930 (69.2)		5520 (304)	
	18	1.01 (0.13)	818 (57.8)		2470 (287)	d	2070 (54.9)		3200 (71.1)		2280 (43.6)		8290 (408)	
Spartina alterniflora	0.5	0.51 (0.04)	739 (23.8)	a	6900 (466)	ab	1920 (136)	a	1760 (103)	a	1130 (30.1)	b	7710 (382)	a
	5.0	1.08 (0.19)	851 (75.7)		7480 (554)	a	2760 (414)		3190 (188)		1930 (82.6)		8230 (741)	
	18	0.49 (0.06)	794 (10.8)		8330 (452)	a	2940 (389)		4020 (271)		2890 (218)		11800 (518)	
Spartina patens	0.5	0.90 (0.24)	541 (59.7)	b	7710 (397)	a	1810 (126)	b	907 (38.8)	c	1150 (44.7)	a	4830 (342)	c
	5.0	0.84 (0.17)	487 (28.8)		4570 (252)	c	1590 (169)		1650 (45.8)		1540 (86.5)		3530 (276)	
	18	0.99 (0.14)	596 (29.9)		4320 (324)	c	2460 (398)		1950 (195)		2640 (265)		6520 (824)	
Treatment [z]		*P* > *F*	*P* > *F*		*P* > *F*		*P* > *F*		*P* > *F*		*P* > *F*		*P* > *F*	
Plant		0.0846	<0.0001		<0.0001		0.0002		<0.0001		0.0132		<0.0001	
Salinity		0.6329	0.1178		0.0023		<0.0001		<0.0001		<0.0001		<0.0001	
Plant × salinity		0.6854	0.2302		<0.0001		0.0069		0.0576		0.0003		0.1775	

312 [z] Least squares analyses of variance (ANOVA); *P* > *F*: The probability of a greater *F* statistic occurring. Based on appropriate ANOVAs, means within columns
313 were separated either as a single treatment (typically plant) or by the interaction term for plant x salinity, when plant responses by salinity differed in trends.
314 Means with the same letter are not significantly different.

315 **Table 4**. Mean (standard error) of micronutrient concentrations in plants harvested at the
316 conclusion of the 7-week experiment.

Plant species	Salinity ($g \cdot L^{-1}$)	Zn ($mg \cdot kg^{-1}$)		Cu ($mg \cdot kg^{-1}$)		Mn ($mg \cdot kg^{-1}$)		Fe ($mg \cdot kg^{-1}$)	
Distichlis spicata	0.5	46.8 (2.82)	bc	9.18 (0.86)	a	35.0 (1.74)	a	402 (81.5)	a
	5.0	35.0 (1.94)		7.13 (0.25)		56.5 (3.75)		619 (123)	
	18	45.8 (2.61)		8.32 (0.44)		169 (21.2)		2251 (258)	
Juncus roemerianus	0.5	37.4 (3.35)	bc	6.87 (0.27)	b	39.1 (4.35)	b	237 (44.9)	c
	5.0	26.2 (0.62)		6.44 (0.46)		39.3 (2.96)		239 (55.0)	
	18	27.2 (1.07)		6.52 (0.36)		54.7 (2.98)		585 (53.7)	
Spartina alterniflora	0.5	60.9 (3.53)	c	5.63 (0.36)	c	41.2 (3.64)	a	293 (20.9)	b
	5.0	61.0 (5.97)		5.58 (0.88)		74.6 (12.5)		837 (312)	
	18	36.5 (4.30)		4.33 (0.51)		106 (14.3)		761 (179)	
Spartina patens	0.5	98.4 (10.4)	a	10.1 (1.15)	ac	35.3 (1.54)	a	289 (134)	ab
	5.0	36.9 (4.50)		5.19 (0.39)		57.0 (4.24)		491 (36.5)	
	18	40.3 (4.70)		6.20 (0.83)		122 (26.8)		1595 (376)	
Treatment [z]		*P* > *F*		*P* > *F*		*P* > *F*		*P* > *F*	
Plant		<0.0001		0.0003		0.0002		<0.0001	
Salinity		0.0002		0.0399		<0.0001		<0.0001	
Plant × salinity		0.0059		0.3210		<0.0001		0.0001	

317 [z] Least squares analyses of variance (ANOVA); *P* > *F*: The probability of a greater *F* statistic occurring. Based on
318 appropriate ANOVAs, means within columns were separated by the plant treatment, as trends for the interaction
319 term or salinity alone were similar (changed in the same manner). Means with the same letter are not significantly
320 different.

321 For all plant species across salinity treatments, similar concentrations ($p = 0.363$) of Ca were
322 measured in plant shoots and roots. However, Ca final concentrations in plant species differed (p
323 0.0003), with *D. spicata* and *S. alterniflora* fixing more Ca by relative concentration ($p =$
324 0.0004) than *S. patens* or *J. roemerianus*. Calcium plays an important role in plant salinity
325 tolerance and is often used in sensing and signaling to induce metabolic adaptations (Seifikalhor
326 et al., 2019). Previous studies have reported that elevated Ca levels can protect plants from Na
327 toxicity and help in homeostasis; however, high Ca concentrations can be toxic if they persist for
328 a long time in the cytosol (Matsumoto et al. 2002; Kader and Lindberg 2010; Seifikalhor et al.
329 2019). *Spartina alterniflora* had the highest concentration of Mg in tissues, followed by *J.*
330 *roemerianus* > *D. spartina* = *S. patens* ($p < 0.0001$).

3.5. Nutrient ratios in plant tissues: initial vs. harvested plants

Ratios of nutrients fixed within plant tissues shifted between those measured in plants initially harvested from the saltmarsh and those grown in FTWs exposed to increasing salinity ($p < 0.0001$; Figure 5). The whole plant ratio of Na:Ca was higher in *D. spicata* ($p < 0.0001$), *J. roemerianus* ($p = 0.0008$), *S. alterniflora* ($p < 0.0001$), and *S. patens* ($p < 0.0001$) grown in the saltmarsh (initial) vs. those grown in the FTWs, across all salinity exposures. For all species, the concentration of sodium vs. calcium present in tissues was higher when plants were grown in soil vs. water, and the Na:Ca ratio for the plants grown in water averaged 2.9 vs. 7.1 for those grown in soil. Despite this apparent increase in Ca concentration in the plants, salinity tolerance was not equally distributed among the species evaluated. Similar trends in lack of correlation between Na:Ca ratio and plant salinity tolerance were noted in Grattan and Grieve (1999).

Interestingly, no consistent trends were evident among species, marsh vs. FTW, and salinity concentration for the remainder of tissue ratios evaluated (Na:K, Mg:Ca, and Na/(Na+Ca); Figure 5, Supplemental Figure 14). The Na:K ratio varied among species, with *S. alterniflora* exhibiting the same trend (higher Na:K ratio in the saltmarsh harvested tissues vs. FTW grown plants). Both *D. spicata* and *S. patens* exhibited similar trends, with the ratio of Na:K in the saltmarsh harvested tissues similar to the 18 $g \cdot L^{-1}$ salinity FTW but differing from the 0.5 and 5.0 $g \cdot L^{-1}$ salinity FTW treatments ($p < 0.0001$). *Juncus roemerianus* differed from all, with the Na:K ratio highest for the 18 $g \cdot L^{-1}$ salinity FTW ($p < 0.0001$). In salinity evaluations, Izzo et al. (1991) and Graifenberg et al. (1995) noted that the K concentration in plant tissues typically declined with increasing Na:Ca ratios. However, Cachorro et al. (1993) noted that Na accumulation increased in concert with leaf K, reflected in a decreasing Na:K ratio with increasing salinity (Cachorro et al. 1993). We noted both trends in partitioning, depending upon the plant evaluated.

354 The Mg:Ca of *J. roemerianus* was lowest in the 0.5 g·L^{-1} salinity FTW treatment in contrast with
355 the other treatments (p <0.0001); whereas the Mg:Ca for *D. spicata* was highest in the saltmarsh
356 harvested tissues vs. the FTW salinity exposures (p <0.0001). For all other species, the Mg:Ca
357 was more variable. In *S. alterniflora* tissues, the Mg:Ca increased with increasing salinity, with
358 the highest Mg:Ca recorded for the saltmarsh (initial) plants. While for *S. patens*, the Mg:Ca
359 ratio was similar and highest (>1.0) for the 5 g·L^{-1} salinity exposure > 18 g·L^{-1} salinity ≈
360 saltmarsh plants (initial) > 0.5 g·L^{-1} salinity treatment. When excess Ca is available in solutions,

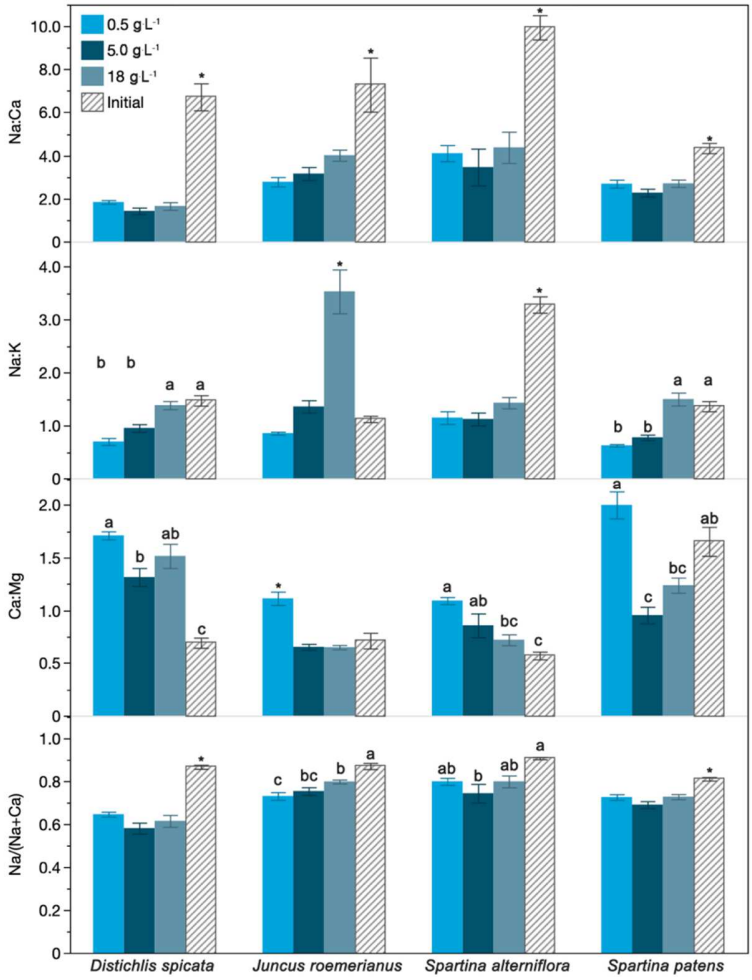

Figure 6. Ratios of Na:Ca, Na:K, Mg:Ca, and Na/(Na+Ca) in plants harvested from the saltmarsh (Initial) and after 7 weeks in FTWs at 0.5, 5.0, and 18 g·L^{-1} salinity. Least squares analyses were conducted within plant species and means with the same letter do not differ. Bars with * indicates the single treatment differed from others within that plant species (p < 0.05).

plants tend to prefer to absorb Ca rather than Mg from solution; this trend is common in high salinity environment and often results in Mg deficiencies in leaf tissues (Grattan and Grieve 1999). Takamoto et al. (2021) also reported potential for reduced growth in soybean if the Mg:Ca was < 1.4 (indicating excess Ca in solution vs. Mg). Other studies have reported Mg deficiency in sesame (Nassery et al. 1979) and blueberry (Bryla et al. 2021) with excess Ca available in solution. Nassery et al. (1979) concluded by stating the importance of multi-salt solutions for salt-tolerance evaluations. But little work has explored how salt mixtures influence salinity tolerance in wetland species, horticultural crops, or plants used in FTWs installed in brackish waters.

4. Conclusions

Growth of saltmarsh plant species evaluated in FTWs varied when exposed to increasing levels of salinity. *Distichlis spicata* and *J. roemerianus* had the lowest biomass production and final nutrient concentrations in tissues. Additionally, *J. roemerianus* did not survive at 18 $g \cdot L^{-1}$ salinity, but plant death might have been a result of age-specific responses to increased salinity or the need for an extended acclimation period. The influence of salinity on survival of younger *J. roemerianus* plants should be further investigated to determine if plant maturity stage influences survival more than salinity exposure. Conversely, *S. patens* and *S. alterniflora* grew well in the oligotrophic water quality of the exposures (0.089 $mg \cdot L^{-1}$ TN, 0.017 $mg \cdot L^{-1}$ PO_4-P) and absorbed nutrients across all salinities. *Spartina patens* showed more stress symptoms and grew less at 5.0 and 18 $g \cdot L^{-1}$ salinity. *Spartina alterniflora* presented few stress symptoms and grew well across all salinity exposures. Of particular interest were changes in nutrient partitioning within tissues, as plants harvested from the salt marsh had different elemental ratios and concentrations fixed within tissues than plants harvested from the salt marsh and then grown in FTWs under varied salinity treatments. Additional research is needed to determine if these findings hold true in eutrophic and hypereutrophic conditions, as the additional nutrients common in anthropogenically impacted brackish systems may enable these species to overcome salinity stress.

Statements and Declarations

CRediT authorship contribution statement.

All authors contributed to study Conceptualization and Design. **Landaverde**: Methodology, Investigation, Writing – Original Draft. **Strosnider**: Methodology, Supervision, Writing- Review & Editing. **White**: Funding, Supervision, Writing – Review & Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability.

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon request.

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