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Halophyte Response to Drought and Salinity Stress and Implications for Restoration

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UNIVERSITY OF CALIFORNIA  
SANTA CRUZ

**Halophyte Response to Drought and Salinity Stress  
and Implications for Restoration**

SENIOR THESIS  
in  
ECOLOGY AND EVOLUTIONARY BIOLOGY

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## **Abstract**

Due to global loss and degradation of salt marshes, restoration and conservation have become necessary to protect and preserve salt marsh systems. Restoration can exacerbate the common environmental stressors – salinity and drought – present in Mediterranean salt marsh ecosystems. Grading and clearing of land to restore tidal influence and remove non-native weeds creates large expanses of bare soil, increasing evaporation rates. As moisture is lost, salts are concentrated in the soil, making growing conditions more difficult for transplants. Multiple species are usually planted during revegetation efforts, but different species are likely to vary in tolerance to moisture and salinity stress; yet, the relative effect of these stressors on many California salt marsh natives are unknown. To address this knowledge gap, we applied greenhouse watering treatments to five perennials common in central California coast salt marshes. We evaluated response to water volume and salinity by measuring survival, growth, and tissue water potential. As predicted, drought significantly reduced tissue water potential for all five species; however, only three species showed a significant decline in water potential with increasing of salinity treatments. Contrary to expectations, growth was unaffected by drought or salinity treatments. Our results suggest these species have broad tolerance to drought and salinity stress they may encounter in the salt marsh ecotone.

## Introduction

Coastal salt marshes are a vital interface between terrestrial and marine ecosystems, providing erosion protection, secure nurseries, runoff filtration, and critical habitat for threatened species (Dyke & Wasson, 2005; Silliman, Grosholz, & Bertness, 2009). Despite the value of these ecological services, most salt marshes have been lost or degraded by human activities (Silliman et al., 2009). In fact, 91% of the wetlands in California have been drained and reclaimed for other uses, and the few that remain exist in altered states (Caffrey, Brown, Tyler, & Silberstein, 2002; Dyke & Wasson, 2005; Fariña, Silliman, & Bertness, 2009; Silliman et al., 2009). To restore ecosystem services lost to these changes requires re-establishment of healthy salt marsh vegetation (Gedan, Silliman, & Bertness, 2009).

Tidal inundation regimes create salinity and moisture gradients that covary with elevation, driving variations in abiotic conditions that can restrict plants to specific zones within the ecotone (Zedler et al., 1999). An ecotone is a transition between two ecological systems with a steep environmental gradient, such as salinity and moisture levels (Wasson, Woolfolk, & Fresquez, 2013). At lower elevation in the ecotone, frequent inundation ensures regular soil saturation and salinity values close to that of seawater, and species intolerant of these conditions are restricted to higher elevations (Fariña, Silliman, and Bertness, 2009). In the upper ecotone tides are infrequent, and in the absence of rainfall, salts are concentrated in the soil via evaporation. In Atlantic coastal salt marsh, year-round rainfall prevents buildup of salts, creating relatively benign growing conditions in the upper ecotone (Bertness & Hacker, 1994; Noto & Shurin, 2017). In contrast, the dry summers of Mediterranean climates drive high evaporation rates that concentrate salts in the soil, making conditions at higher elevations more stringent during warmer parts of the year (Callaway, Jones, Ferren Jr., & Parikh, 1990; Fresquez, 2014).

Due to the elevation gradient, tidal regimes, and variable rainfall, salt marsh ecotones develop salinity and moisture zones that can intersect in ways that impact species differently. Change within these zones can be driven by environmental factors as well as human influence, and impose short or long-term effects; for example, heavy rain can temporarily dilute the salinity gradient, while the breaching of a dike can restore a salinity gradient where it has long been absent (Pennings & Callaway, 1992; Pennings, Grant, & Bertness, 2005). Salt marsh systems therefore have dynamic abiotic gradients that can be further modified through restoration activities. Removal of non-native species creates large bare soil patches, where higher evaporation rates concentrate salts in the soil (Bertness & Hacker, 1994; Bertness & Shumway, 1993; Callaway, 1994). Restoration activities can thereby intensify naturally occurring moisture and salinity gradients present, affecting success of salt marsh revegetation efforts. If the relative influence of these stressors varies across species, planting strategies that account for these differences could improve restoration outcomes. This knowledge would facilitate planting species into zones where stress levels are tolerable. For instance, *Distichlis spicata* can tolerate a wide range in salinity, but little is known about its sensitivity to drought (Kemp & Cunningham,

1981). Intelligent placement of *D. spicata* might therefore depend on its water requirement rather than salinity limitation.

Salinity affects water uptake, transport, and transpiration, requiring plants to have adaptations to survive in saline soils (Grigore & Toma, 2017a; Reimold, 1974). Salt secretion via salt glands is the most common method of salt removal for non-succulent plants; however, salt can also be removed via salt hairs. Both methods require the plant to take up salt and eliminate it through specialized organelles (Grigore & Toma, 2017a). Another common strategy is succulence, which dilutes ions to non-lethal levels, allowing plants to survive in high salinity environments (Grigore & Toma, 2017b). Salinity exclusion in the roots is a third method, though it is much less common (St. Omer & Schlesinger, 1980c). Soil moisture also affects plant performance, because water uptake, photosynthesis, and turgor pressure can be reduced under dry or high salinity conditions (Grigore & Toma, 2017b; Jong, 1978; Reimold, 1974; St. Omer & Schlesinger, 1980a). Both low soil moisture and high soil salinity can decrease plant water potential, which is measured as the negative pressure required to move water through the plant. The lower the value, the more difficult it is to take up and move water through the plant (T J Flowers, P F Troke, & Yeo, 1977; Taiz, Zeiger, Moller, & Murphy, 2015), possibly affecting growth and survival (T J Flowers et al., 1977; Taiz et al., 2015). For this reason, water potential is often used as an indicator of stress.

Here, we applied watering treatments varying in salinity and volume to determine the relative influence of each on halophyte plant performance. We expected to observe a more negative water potential for plants in drought or high salinity treatments compared to plants in saturation or freshwater treatments. In addition, salinity and drought stress should exhibit interactive effects, such that combinations of moderate salinity and drought also reduce performance. We predicted that plant tissue water potential would reflect stress caused by drought and/or salinity, and that more negative values would correlate with reduced survival and growth. Because the natural distribution of salt marsh species differs within the ecotone, where moisture and salinity covary with elevation, we expect treatment effects to vary across species. To test these hypotheses, we subjected five native perennials to eight different watering treatments in the greenhouse. Plant tissue and soil water potential were measured to assess physiological and abiotic effects of treatments, and growth and survival were tracked to assess treatment effects on plant performance.

## **Methods**

### **Species and Site Description**

Elkhorn Slough is a tidal wetland connected to Monterey Bay (Monterey County, California). It contains salt marshes, channels, eelgrass beds, mud flats, various upland environments, and freshwater streams. The connection to Monterey Bay provides tidal movement necessary for salt marsh formation (Dyke & Wasson, 2005). Elkhorn Slough experiences a Mediterranean climate with average temperature ranging from 11.1°C in the winter to 15.4°C in the summer, and mean annual rainfall of 55.2 cm, falling primarily during the

winter months. There is also a pattern of interannual rainfall with droughts lasting from two to six years. During spring and summer dense fog is common, with humidity averaging around 80% (Caffrey et al., 2002). Elkhorn Slough is a vital breeding and nursery ground for numerous species of birds and fish, as well as two species of marine mammals. The low marsh vegetation is dominated by *Salicornia virginica*, while *D. spicata*, *Frankenia salina*, *Extriplex californica*, *Jaumea carnosa* (Caffrey et al., 2002; Reimold, 1974), and *Spergularia macrotheca* are common perennial natives of the high marsh. *D. spicata* (Poaceae) is a salt marsh grass that removes salt through specialized salt glands in the leaf blades (Hansen, Dayanandan, Kaufman, & Brotherson, 1976; Reimold, 1974). *E. californica* (Chenopodiaceae) is a woody dicot that uses salt bladders to extrude excess salt (Grigore & Toma, 2017a). *F. salina* (Frankeniaceae) is an herbaceous, woody halophyte with salt removal glands embedded in its leaves (Grigore & Toma, 2017a; Levering & Thomson, 1971). *J. carnosa* (Asteraceae) is an herbaceous, succulent halophyte that grows exclusively on the Pacific coast. Its optimal soil salinity level is within the medium to low range, but the species has high salinity tolerance in hydroponic cultures (Grigore & Toma, 2017b; St. Omer & Moseley, 1981a; St. Omer & Schlesinger, 1980a). *S. macrotheca* (Caryophyllaceae) is a woody halophyte. Observed elevation ranges for *D. spicata*, *F. salina*, and *J. carnosa* partially overlap, with *D. spicata* extending furthest upland, and *J. carnosa* extending lowest in the marsh (Fresquez, 2014; Wasson et al., 2013).

### **Plant propagation**

Seeds of *E. californica*, *F. salina*, *J. carnosa*, and *S. macrotheca* were collected from the Elkhorn Slough watershed during spring and summer 2016. Seeds were germinated in the UCSC Greenhouse in fall 2016, after sowing into stubby containers (Stuewe & Sons, Ray Leach Containers model SC7) filled with Premiere Horticultural ProMix Hp potting soil. Once established, seedlings were moved into an outdoor area. *D. spicata* was supplied by Elkhorn Native Plant Nursery (now defunct), with plants grown in 2" rose pots using a custom soil mix. All plants received water as needed prior to initiation of the experiment.

### **Preliminary Experiments**

To create a gradient of moisture and salinity stress in the greenhouse, we conducted three preliminary experiments. The first and second identified watering volumes that would induce drought stress but avoid total mortality. The first identified a range of possible watering volumes through soil moisture retention while the second identified final watering volumes using *D. spicata* and *S. macrotheca*. The third preliminary experiment developed treatments that imposed a gradient of salinity stress while avoiding mortality (Appendix 1, Table 5, Table 6). To do so, we applied five watering treatments to *D. spicata* and *J. carnosa*, allowing us to observe the effects of different watering volumes and salinity concentrations on plants.

### **Watering treatments**

We collected seawater from Cowell's Beach (Santa Cruz, California) and stored it in plastic jugs for less than one week before use. We mixed seawater with deionized (DI) water to create three dilutions: 30% seawater (-1.45 MPa), 45% seawater (-1.72 MPa), and 60% seawater

(-2.17 MPa). Undiluted seawater had a water potential of -2.89 MPa. The control was DI water (0 MPa). The plants in conetainers (*F. salina*, *J. carnosus*, *E. californica*, and *S. macrotheca*) received drought treatments of 15 mL or saturation treatments of 40 mL. *D. spicata* in rose pots received drought treatments of 15 mL or saturation treatments of 35 mL. All plants were watered individually using a 25 mL  $\pm$  1mL serological pipette (SEOH). Due to soil compaction in soil blanks, we squeezed these conetainers weekly to disrupt the soil and facilitate drainage.

### **Greenhouse experiment setup**

To maintain conetainers as experimentally independent units, we inserted individual conetainers in 8 oz Styrofoam cups (Appendix 2). All cups were positioned on mesh greenhouse tables that drained excess water away, preventing the treatment applied to any conetainer from affecting neighbors. We randomly assigned plants (N = 122, Table 1) to treatments and an initial position on the greenhouse table, systematically shifting plants weekly to compensate for variations in greenhouse conditions. Soil blanks (N = 112) were also assigned to treatments and randomly intermixed with each of the 5 species.

### **Plant tissue and soil collection**

Because the tissue measuring process was time-intensive, we harvested two species at a time to minimize drying of samples. We harvested *F. salina* and *D. spicata* tissue at eleven weeks for water potential testing; these species were processed first due to elevated mortality (*D. spicata* mortality was due to desiccation, while *F. salina* was experiencing heavy aphid attack). At thirteen weeks, *J. carnosus* and *E. californica* were harvested. *S. macrotheca* and soil blanks were harvested at the beginning of the fourteenth week. Although we see no evidence of systematic bias resulting from staggered harvest, we cannot rule out the possibility of an effect. Following tissue harvest for each species, we cut green stems and leaves into 0.5 – 1.0 cm lengths before placing them into 15 mL sample cups (AquaLab). We immediately placed lids on cups and wrapped stacks of four cups with Parafilm “M” (American National Can) to prevent moisture loss. We stored tissue samples in a cool, dry place for a maximum of three days before processing, and we randomized processing order among treatments to avoid biases related to length of storage time. We emptied soil blanks into 1-quart Ziploc bags and sealed them inside a second bag. Soil was homogenized inside bags before dispensing into sample cups. Soil samples were stored in Ziploc bags for approximately one month before processing, due to technical issues with our instrument.

### **Water potential measurements**

We used a WP4 Dewpoint PotentiaMeter (Meter Group, Inc.) to measure water potential of plant tissue and soil samples. We also used the Dewpoint PotentiaMeter to measure the water potential of the four treatment solutions – freshwater, 30% seawater, 45% seawater, and 60% seawater – confirming salinity differences among solutions. Prior to processing, we calibrated the Dewpoint PotentiaMeter with 0.5 molal potassium chloride (KCl) AQUALAB standard (Meter Group, Inc.). Sample cups were filled below the halfway point, and we wiped down the

outside and inner lip of cups with a clean Kimwipe (Kimberly-Clark Professional) before insertion into the WP4 to prevent sensor contamination.

### **Growth measurements**

We recorded survival and length, every four weeks for all species. All stems longer than 3 cm were counted for *J. carnosa*, *F. salina*, *D. spicata*, and *E. californica*, and stem lengths were measured to the nearest 0.5 cm from soil surface to the tip of the stem. Survival was measured for all species. Growth or survival for *S. macrotheca* could not be reliably assessed; stems that appeared to be completely dead sometimes produced green tissue or flowers several weeks later. Entire plants were classified as dead if no green tissue remained. *F. salina* and *E. californica* frequently attracted aphids in the greenhouse, with *F. salina* particularly susceptible to attack. We therefore tracked aphid presence, and we lost many *F. salina* plants to aphid infestation just prior to our second round of growth measurements. Because we could not reliably attribute death of these stems to aphids, we recorded length of dead stems to capture pre-death growth.

### **Statistical analyses**

We modeled plant tissue water potential, soil water potential, and plant growth using two-way ANOVA models including salinity, water volume, and their interaction as fixed effects. Statistical models were built using the base package in R (R 3.4.2, R Core Team 2016) and RStudio 1.0.153 (RStudio Inc., 2009 - 2017).

## **Results**

### **Water Potential**

Soil water potential was affected by treatment, leading to significantly more negative water potentials in the drought treatment, and in treatments of increasing salinity (Fig. 1, Table 2). There was also a significant interaction between drought and salinity (Table 2), with the effects of salinity intensifying in the drought treatment (Fig. 1).

Patterns for tissue water potential were similar with water potential generally declining as salinity increased across all five species (Fig. 2). Although plants in the drought treatment received less than half of the water than the saturation treatment, tissue water potential remained similar across watering volume for most species. Nonetheless, drought had a significant effect on tissue water potential for all five species (Table 2). The effect of salinity and its interaction with drought differed across species; *E. californica* showed a significant response to salinity and the interaction between drought and salinity. *F. salina* and *J. carnosa* showed a significant response to salinity, but not the interaction between drought and salinity. Finally, *D. spicata* and *S. macrotheca* did not respond to either salinity or the interaction between drought and salinity. The range of measured tissue water potential varied greatly among species, with *D. spicata* reaching as low as -12 MPa. In contrast, *J. carnosa* and *S. macrotheca* stayed within -1.5 to -3 MPa, and *E. californica* and *F. salina* had intermediate values.



## Growth Measurements

Watering treatments had no effect on plant growth – neither salinity or drought had a significant effect on any of the species tested (Table 3, Fig. 3). The interaction of drought and salinity was marginally significant for *E. californica*, with negative effects of drought on growth manifesting only when saline water was used.

## Survival

Mortality did not appear to be affected by drought or salinity treatment (Table 4). Mortality was minimal for *D. spicata* and *S. macrotheca*, with more than 93% of individuals surviving to the end of the experiment. Observed *D. spicata* mortality may have resulted from increased drainage through sandy soil in rose pots. *S. macrotheca* stems are brittle and observed mortality resulted from stem breakage during survival surveys. *F. salina* experienced the highest mortality, with 37 individuals lost to aphid attack. It is notable that the highest mortality rate was observed in the drought, 60% seawater treatment, where only 47% of *F. salina* individuals survived. There was no mortality for *J. carnososa* or *E. californica*.

## Discussion

Our experiment simulated two stressors – drought and salinity – that are important determinants of plant distribution in California coastal wetlands. The treatments resulted in distinct water potential patterns for both soil and plant tissue across species. Soil water potential in particular showed a striking response to treatment, with measurements ranging from ~ -0.5 MPa to -6 MPa – low enough to expect impacts on plant performance. The soil water potential at which plants are unable to take up sufficient water to compensate stomatal water loss is known as the permanent wilting point; it is often the soil water potential where the plant irreversibly wilts and dies (Warrick, 2001). -1.5 MPa is commonly accepted as the permanent wilting point for glycophytes (salt intolerant plants); however, Warrick (2001) notes that due to the substantial variation in plant species tolerance, some can survive well past the -1.5 MPa permanent wilting point threshold. At the moment, very little research has been done to identify soil water potential thresholds affecting halophyte performance or permanent wilting points. Treatments here clearly affected plant tissue water potential, with readings ranging from -1.5 MPa to -12 MPa; most species remained above -7.5 MPa. For glycophytes, plant tissue water potential of -1.5 MPa generally reduces cell expansion, cell wall synthesis, and protein synthesis. As water potential continues to drop, photosynthesis and stomatal conductance dramatically decrease, while solute and abscisic acid accumulation increases (Hsiao & Acevedo, 1974). Hsiao and Acevedo (1974) briefly discuss halophytes, but there is insufficient research to draw firm conclusions on halophyte physiological response to decreasing plant tissue water potential. Although the patterns were similar for plant tissue and soil, the magnitude of change was different, so soil water potential cannot be used as a direct indicator of plant tissue water potential.

There was a general pattern that suggested increasing salinity led to decreasing tissue water potential. However, only *E. californica*, *F. salina*, and *J. carnososa* showed a significant

change in water potential as salinity increased. The observed change was likely due to increased solute concentration in the tissue to compensate for higher solute concentration in the soil (Grigore & Toma, 2017b; Reimold, 1974). As soil solute concentrations increase, it becomes more difficult for plants to take up water. In response, plants can concentrate solutes in their tissue, creating a hypertonic state that allows continued passive uptake of water (Grigore & Toma, 2017b, 2017a; Hsiao & Acevedo, 1974).

Lack of response to increasing salinity for some species may have resulted from insufficiently stringent treatments. Our highest salinity treatment was 60% seawater, whereas plants in the low ecotone can experience inundation with full-strength seawater. The average low marsh soil has a salinity concentration of 43.9 ppt (Fresquez, 2014), while seawater averages about 34.9 ppt, indicating that some species can survive 125.79% seawater. Because our plants only received 60% seawater, or roughly half the concentration plants can experience in the field, it would be useful to repeat this greenhouse experiment with higher salinity treatments.

Drought effects can be similar to salinity effects, causing plants to become hypertonic to increase water uptake (Taiz et al., 2015). Drought significantly affected all species, causing a decrease in water potential when compared to the saturation treatment. It should be noted that although our drought treatment had a significant effect, it is unlikely to replicate true field conditions. To avoid mortality in the greenhouse, plants received water every three to four days, which differs greatly from natural rainfall patterns, even in the wet season. During the dry season, soil moisture is often between 30% - 80% of sample weight for marsh and low ecotone soil cores in the high ecotone and upland locations, soil moisture accounts for 0% - 30% of sample weight. We have no data showing whether we achieved similar conditions with potting soil in the greenhouse, and regardless, we would expect more rapid drying in pots than for *in situ* field soil. Thus, as for all greenhouse studies, results presented here should be used with caution when predicting performance in the field. To expand on these results, the greenhouse experiment should be repeated using native marsh soil as the substrate and including higher salinity treatments (e.g. full seawater). Response to treatment in marsh soil should provide a more accurate prediction of response to field conditions.

Surprisingly, measured differences in water potential did not translate to plant performance. Neither growth nor survival were visibly affected by watering treatment, even in potentially stressful low volume / high salinity treatments. Existing literature suggests that halophytes concentrate solutes to generate low tissue water potential, allowing continued passive uptake of water. In this case, low tissue water potential is not detrimental, since it prevents or reduces water deficits that can impair growth. Another possible reason for the lack of effect on growth was timing of the experiment. We began the experiment in June, when most individuals were beginning to reproduce. Beyond this point, energy is less likely to be allocated to vegetative growth and more likely to be allocated towards reproduction or survival strategies, like salt management (Taiz et al., 2015). In contrast, younger plants allocate the majority of their energy to vegetative growth (Munns, 2002). Adaptations, such as salt glands or specialized vacuoles, are

energy expensive and require energy normally allocated to growth (Munns, 2002). Additionally, decreasing water potential has been shown to inhibit cell expansion (Hsiao & Acevedo, 1974), which would disproportionately affect young plants, since the rate of cell expansion in mature plants is reduced. Therefore, by better aligning the experimental period with the natural growth period, and focusing on young plants, treatment effects on growth might become more apparent.

*D. spicata* displayed the greatest variability in tissue water potential, and this variability may have been influenced by factors other than watering treatment. *D. spicata* was grown in shallower, wider pots in a sandier potting medium. In both volume treatments, water would drain quickly through the pots, leading to uneven soil saturation that likely affected treatment efficacy and making it difficult to draw definitive conclusions regarding the large range in water potential. However, low water potential values are not uncommon for *D. spicata*. Other authors have observed sustained, highly negative water potential used to compensate for soil salinity (Kemp & Cunningham, 1981). The highest *D. spicata* mortality in our experiment occurred in the drought treatments, with three out of four deaths in the 60% seawater drought treatment. Nonetheless, increased drainage and evaporation rates likely contributed to mortality for this species.

*E. californica* was affected by both the drought and salinity treatments, causing lower water potential and a slight negative effect on growth. Interestingly, our results contrast with those from another study. Jong (1978) measured *E. californica* net dry weight when irrigated with a saline Hoagland solution in sandy soil, using artificial sea salt instead of seawater. The water potential of their maximum salinity treatment was similar to our 60% seawater treatment, but the authors found that dry weight of *E. californica* decreased significantly as salinity increased. This experiment used young *E. californica* seedlings - the first tissue harvest occurred when seedlings were one month old and continued every 8 days until all plants were harvested, with the authors noting a difference in dry weight between treatments (Jong 1978). Since we did not observe a difference in above ground biomass, the contrasting results may be due to the misalignment of experiment start time with the natural growth period.

*F. salina* did not show an effect of salinity and drought stress on total plant growth, since biomass was maintained across treatments. In contrast, Barbour's and Davis's results showed a decrease in *F. salina*'s growth as salinity increased, with total mortality at approximately 89% seawater Hoagland solution (Barbour & Davis, 1970). Plants in their non-saline control showed the most growth, measured by the length of the main and lateral shoots (Barbour & Davis, 1970). The majority of our plants remained constant in size. The high mortality rate across treatments was driven by aphid infestation, despite attempts to control aphids with Botanigard (BioWorks, Inc.). The highest mortality occurred in the drought, 60% seawater treatment, suggesting that stringent growing conditions may have made plants more susceptible to aphid-induced mortality.

*J. carnososa* was the only species that added biomass between the first and final surveys. However, growth did not differ across treatments (Fig. 5). Other studies have found mixed

effects of salinity and drought treatments on growth. One study found that *J. carnosa* grew best in non-saline or minimal saline environments (0% seawater or 11% seawater), using recently germinated individuals with stalks that extended 1-10cm above the growing substrate (Barbour & Davis, 1970). In contrast, two other studies found that *J. carnosa* can tolerate salinities twice as concentrated as seawater, but moderate salinity conditions were ideal (St. Omer & Moseley, 1981b; St. Omer & Schlesinger, 1980b). St. Omer and Schlesinger (1980b) used Hoagland solution in a greenhouse experiment to determine that maximum *J. carnosa* growth, measured by total dry weight, occurred at about 30% - 60% NaCl, with growth decreasing above 60% salinity. They did not record plant age (St. Omer & Schlesinger, 1980b). The age of the plants likely impacted the differences in growth among studies due to the difference of energy allocation between mature and immature plants, which would have been exacerbated with higher salinity. Barbour and Davis used younger plants, which may have been more sensitive to treatment effects compared to the St. Omer and Schlesinger experiment (1980b), and the results reported here. Our experimental results align more closely with those of St. Omer and Schlesinger (1980b), even though our experimental design was more similar to Barbour and Davis (1970).

The experiment should also be repeated with younger plants to determine if age has any effect on salinity and drought tolerance. Other experiments that used younger plants observed a decrease in growth or total biomass as salinity levels increased, contrasting with our finding that plants are largely unaffected by salinity. Seedlings are more desirable to use in revegetation operations due to the reduced propagation cost and transplant effort, so it is important to determine the range of conditions young plants can tolerate.

Our experiment addressed a knowledge gap regarding halophyte salinity and drought tolerance that could inform the design of future restoration projects and experiments in Pacific coast salt marshes. Revegetation efforts often have low success rates due to the stringent abiotic conditions within the ecotone, which disproportionally affect seedlings (Shumway & Bertness, 1992; Williams & Faber, 2001; Zedler et al., 1999). Furthermore, the different natural distributions of halophytes within the ecotone suggest that salinity and drought tolerance could vary among species. In our experiment, treatments had negligible effects on growth or survival – only water potential was affected. These results imply that these five species could survive anywhere within the ecotone, by employing different physiological adaptations – such as succulence, salt glands – to withstand stressful conditions. However, our results are likely not representative of plant performance in the field due to a variety of factors. The timing of our experiment did not align with the natural growth period of the plants, causing us to use mature plants rather than young seedlings. Additionally, our use of 60% seawater is not representative of the tidal inundation that some of the species may experience in the field. Therefore, future experiments will examine how these factors influence outcomes, using lessons learned during this effort. Taken together, findings from this set of experiments will allow us to 1) identify zones within the ecotone maximizing survival and establishment on a by-species basis, or 2) demonstrate that species are flexible enough to compensate for conditions across the ecotone,

making careful placement of species unnecessary. In either case, these experiments will provide valuable insight to restoration practitioners. Ultimately, we hope that this work will support rapid and robust strategies to recreate thriving salt marsh systems.

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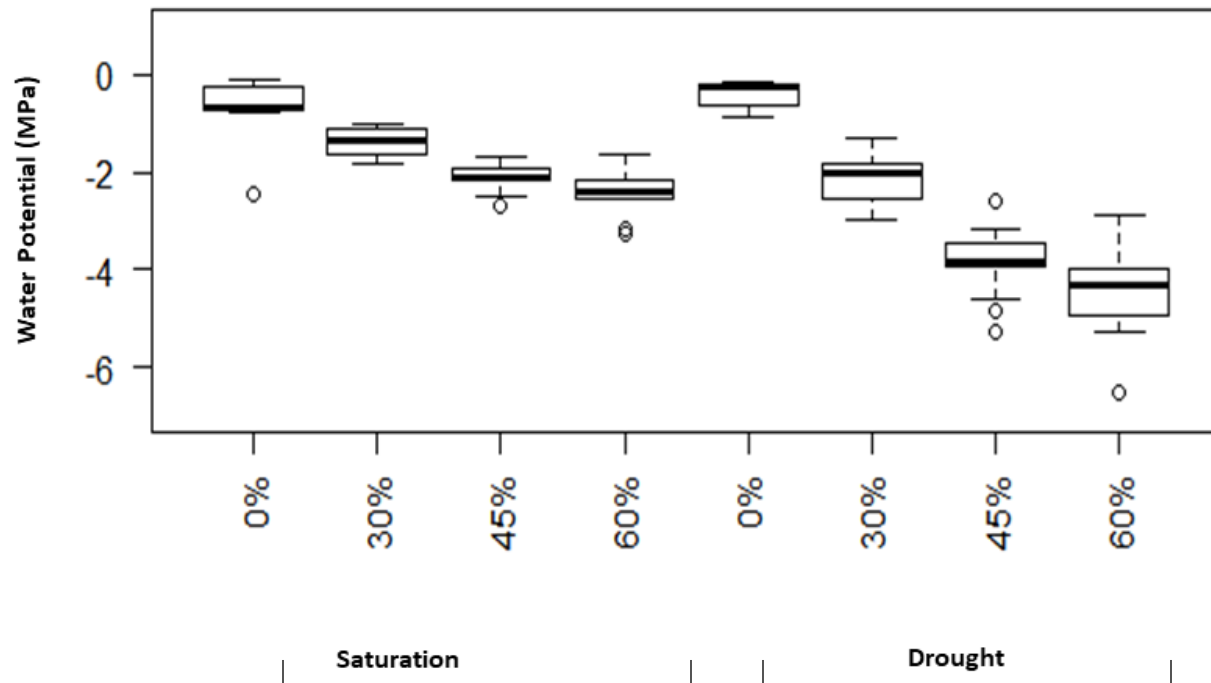
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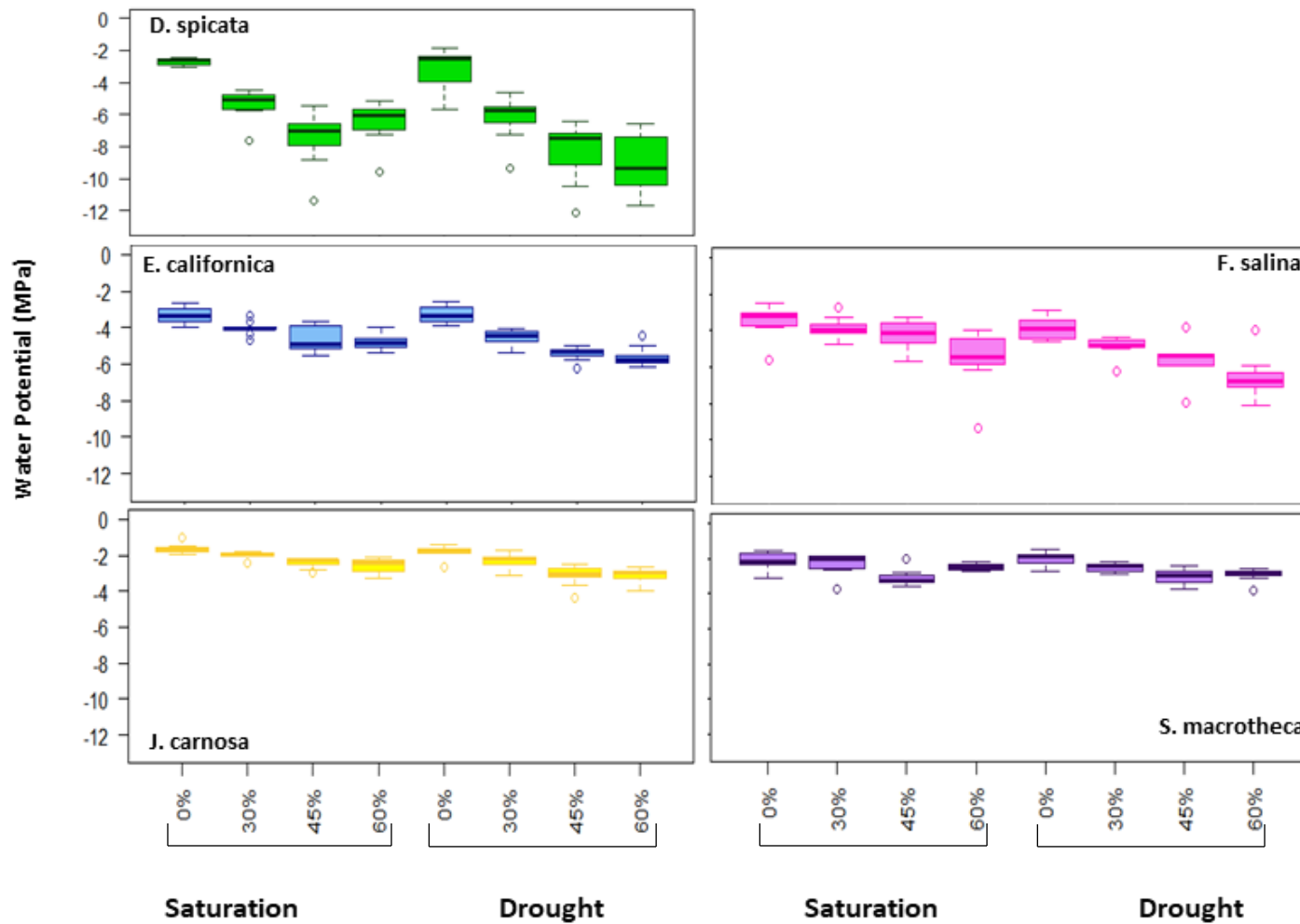
## Figures

*Table 1: Sample Sizes Across Salinity Treatments*

	0% Seawater	30% Seawater	45% Seawater	60% Seawater
<i>D. spicata</i>	N=13	N=16	N=16	N=16
<i>E. californica</i>	N=13	N=16	N=16	N=16
<i>F. salina</i>	N=13	N=16	N=16	N=16
<i>J. carnosa</i>	N=13	N=16	N=16	N=16
<i>S. macrotheca</i>	N=13	N=16	N=16	N=16



**Figure 1:** Soil water potential resulting from saturation and drought treatments ranging in salinity from fresh water to 60% seawater. X-axis shows the salinity treatments grouped by water volume treatment.

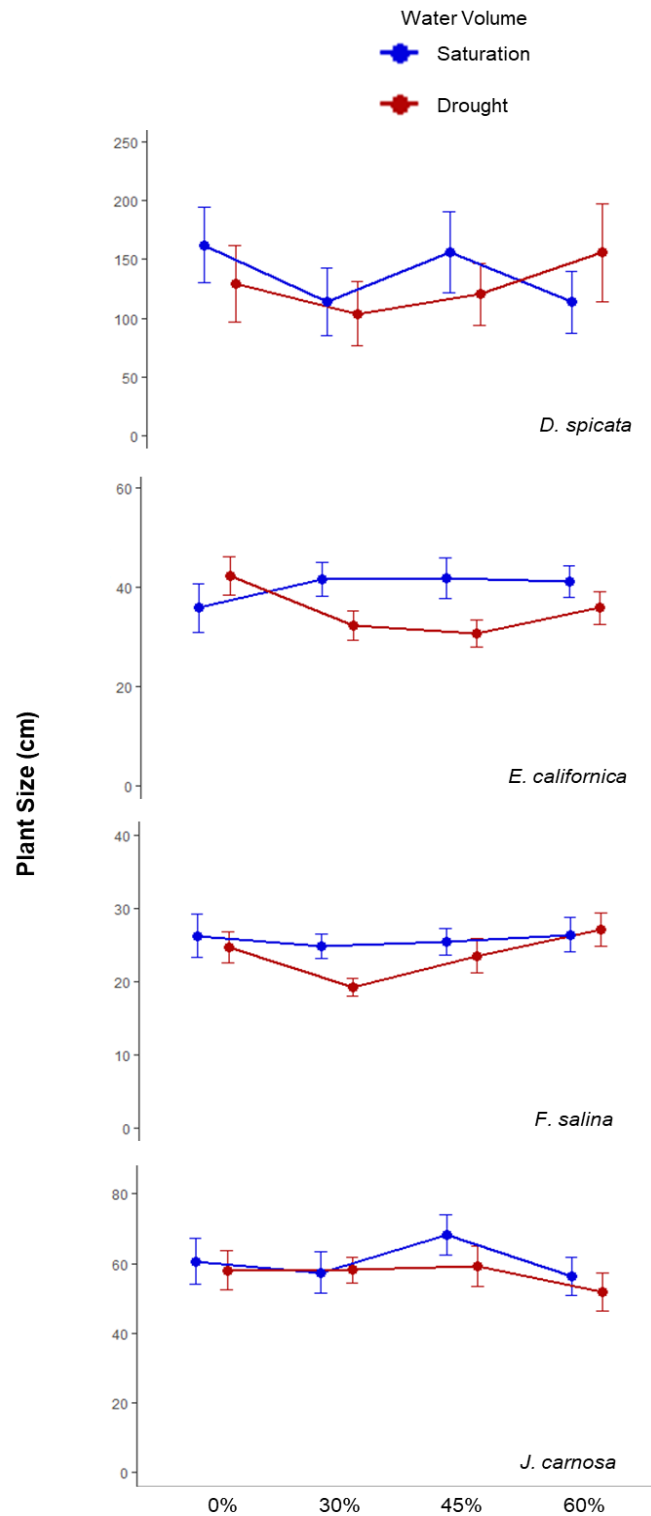


**Figure 2:** Tissue water potential of five species after applying saturation and drought treatments ranging in salinity from fresh water to 60% seawater

*Table 2: Results from Analysis of Variance for Water Potential*

	Treatment	Degrees of Freedom	F-value	p-value
Soil	Drought	3	173.853	<b>&lt;0.001</b>
	Salinity	1	109.177	<b>&lt;0.001</b>
	DroughtxSalinity	3	23.913	<b>&lt;0.001</b>
<i>D. spicata</i>	Drought	3	13.3089	<b>&lt;0.001</b>
	Salinity	1	1.1460	0.288
	DroughtxSalinity	3	0.0705	0.976
<i>E. californica</i>	Drought	3	72.5481	<b>&lt;0.001</b>
	Salinity	1	26.3286	<b>&lt;0.001</b>
	DroughtxSalinity	3	3.7138	<b>0.015</b>
<i>F. salina</i>	Drought	3	13.1433	<b>&lt;0.001</b>
	Salinity	1	10.7133	<b>0.002</b>
	DroughtxSalinity	3	0.5118	0.676
<i>J. carnosa</i>	Drought	3	40.470	<b>&lt;0.001</b>
	Salinity	1	28.4067	<b>&lt;0.001</b>
	DroughtxSalinity	3	2.2501	<i>0.090</i>
<i>S. macrotheca</i>	Drought	3	11.2812	<b>&lt;0.001</b>
	Salinity	1	0.9089	0.345
	DroughtxSalinity	3	1.0154	0.394

Significant values shown in bold and marginally significant values shown in italics.



**Figure 3:** Final plant size for each species after undergoing saturation and drought treatments using a range of salinities. Final plant size was determined by adding all measured stem lengths for each individual plant and calculating the total plant size for each treatment. Growth measurements were not taken for *S. macrotheca*. Error bars are one standard error.

<i>Table 3: Results from Analysis of Variance for Final Plant Size</i>				
	Treatment	Degrees of Freedom	F-value	p-value
<i>D. spicata</i>	Drought	3	0.5092	0.677
	Salinity	1	0.1298	0.719
	DroughtxSalinity	3	0.6674	0.574
<i>E. californica</i>	Drought	3	0.2314	0.874
	Salinity	1	4.5686	0.347
	DroughtxSalinity	3	2.2655	0.085
<i>F. salina</i>	Drought	3	1.5848	0.197
	Salinity	1	1.5597	0.214
	DroughtxSalinity	3	1.0293	0.383
<i>J. carnosa</i>	Drought	3	1.5066	0.215
	Salinity	1	0.6201	0.433
	DroughtxSalinity	3	0.6001	0.616

Italic text indicates marginal significance.



Table 4: Survival Across all Species and Treatments

		Saturated, 0% seawater	Saturated, 30% seawater	Saturated, 45% seawater	Saturated, 60% seawater	Drought, 0% seawater	Drought, 30% seawater	Drought, 45% seawater	Drought, 60% seawater
<i>D. spicata</i>	Survey 1	100%	100%	100%	100%	100%	100%	100%	100%
	Survey 2	100%	94%	100%	100%	100%	100%	100%	93 %
	Survey 3	100%	94%	100%	100%	100%	100%	100%	93%
<i>E. californica</i>	Survey 1	100%	100%	100%	100%	100%	100%	100%	100%
	Survey 2	100%	100%	100%	100%	100%	100%	100%	100%
	Survey 3	100%	100%	100%	100%	100%	100%	100%	100%
<i>F. salina</i>	Survey 1	100%	100%	100%	100%	100%	100%	100%	100%
	Survey 2	85%	75%	88%	94%	77%	100%	80%	82%
	Survey 3	69%	69%	69%	94%	69%	80%	60%	47%
<i>J. carnosa</i>	Survey 1	100%	100%	100%	100%	100%	100%	100%	100%
	Survey 2	100%	100%	100%	100%	100%	100%	100%	100%
	Survey 3	100%	100%	100%	100%	100%	100%	100%	100%
<i>S. macrotheca</i>	Survey 1	100%	100%	100%	100%	100%	100%	100%	100%
	Survey 2	100%	94%	94%	100%	100%	100%	100%	100%
	Survey 3	100%	94%	94%	100%	100%	100%	100%	100%

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*Table 5: Watering Volume and Frequency for All Species*

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	Saturation Treatment	Drought Treatment
Conetainers	40 mL	15 mL
Rose Pots	35 mL	15 mL

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All treatments were applied twice weekly using the specified water volume.

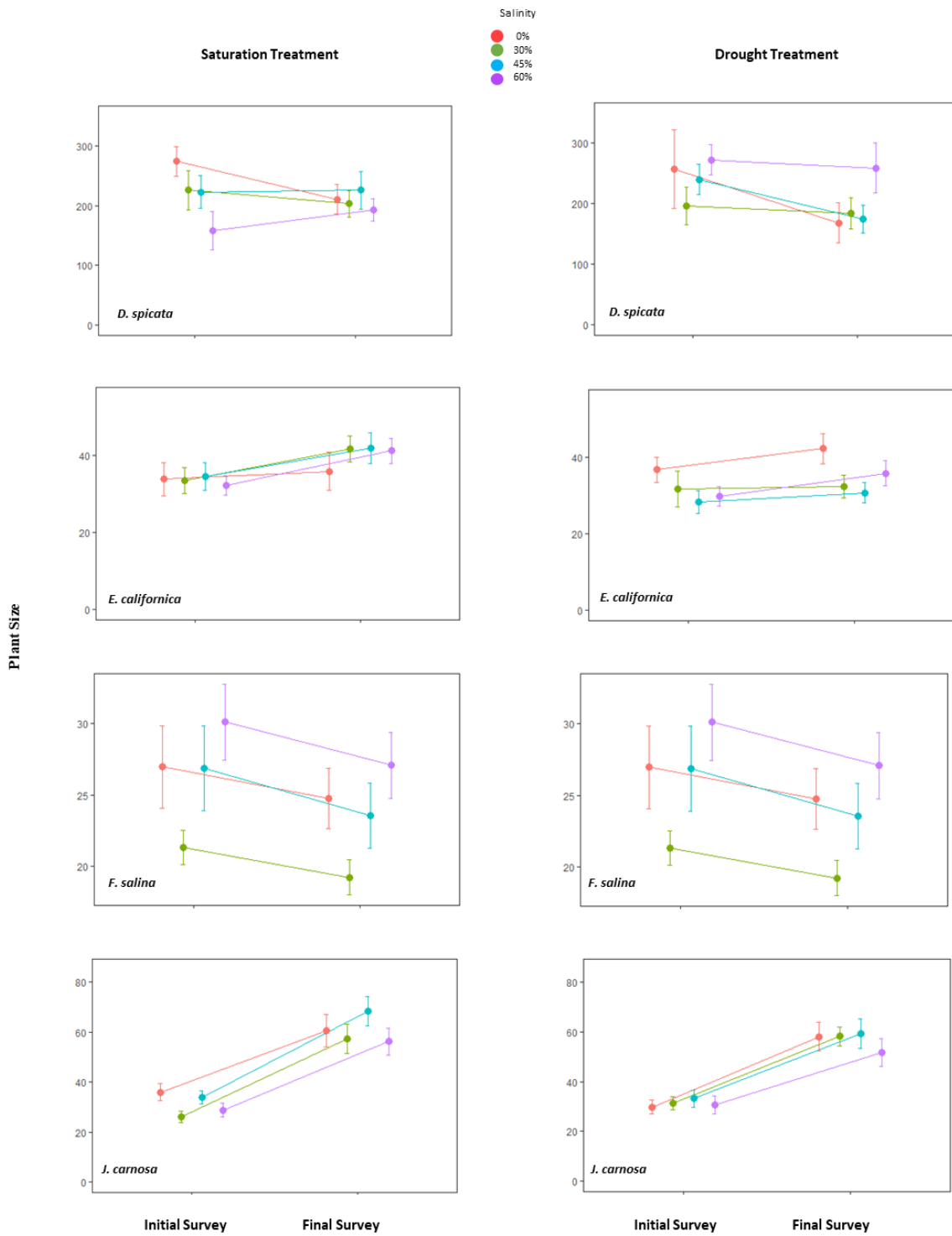
*Table 6: Preliminary Experiment: Salinity and Water Volume*

Species	Water Volume (mL)	Percent seawater (%)
<i>J. carnosa</i>	15	30
	15	60
	40	30
	40	60
	>40	DI water
<i>D. spicata</i>	15	30
	15	60
	35	35
	35	60
	>35	DI water
Soil blanks (conetainers)	15	30
	15	60
	15	DI water
	40	30
	40	60
	40	DI water

All treatments were applied twice weekly using the specified water volume.



**Figure 4:** Labeling and arrangement of containers and Styrofoam cups on greenhouse tables.



**Figure 5:** Initial and final plant size for each species in drought (right column) and saturation (left column) treatments using a range of salinities. Initial and final plant sizes were determined by adding all measured stem lengths for each individual plant. Error bars are one standard error.

## Appendix

### Appendix 1 –Development of Watering Treatments

In a preliminary experiment, we identified the volume of water needed to saturate soil in conetainers (Stuewe & Sons, Ray Leach Cone-tainers model SC7) and 2” rose pots. We first watered containers packed with damp potting soil (Premiere Horticultural ProMix Hp) until water readily drained from the bottom. To this volume we added 5 mL to ensure complete soil saturation, arriving at a target saturation volume of 40 mL for conetainers and 35 mL for rose pots. Four additional conetainers filled with soil were watered with 30 mL, 20 mL, and 10 mL and placed under ambient conditions in the greenhouse. We checked soil at the top and bottom of conetainers every two days for a week to monitor rate of moisture loss. The 40 mL and 30 mL treatments were sufficient to keep soil damp for up to five days. The 10 mL treatment did not completely saturate the soil and allowed it to fully dry between treatments.

To determine how watering treatments affected plants, we set a watering schedule using the water volumes described above. Due to limited plant stock, we tested two species – the grass *D. spicata*, and the herbaceous perennial *S. macrotheca*. Eight *S. macrotheca* individuals in conetainers were watered with 30 mL, 20 mL, 10 mL, or 5 mL of fresh water. Half of the individuals were watered twice a week, and half were watered once a week. Similarly, eight *D. spicata* individuals in rose pots were given 35 mL, 20 mL, 15 mL, or 10 mL of fresh water; half were watered twice a week, and half were watered once a week. Plants in the 5 and 10 mL treatments experienced wilting and leaf death. Plants receiving above 20 mL remained healthy. We therefore settled on twice weekly watering using 40 mL or 15 mL for conetainers, and 35 mL or 15 mL for rose pots (Table 5).

Next, we conducted a preliminary experiment to test the effects of treatments on water potential of soil and plant tissue, using the water volumes determined above (Table 5). Due to limited plant stock, we tested two species - *D. spicata* (2” rose pots) and *J. carnososa* (conetainers), as well as soil blanks in conetainers. We had five treatments, with six individuals of *J. carnososa* and *D. spicata* per treatment (Table 6). A DI water saturation treatment (greater than 40mL) was applied as the control, and effects of drought and salinity treatments were assessed against this baseline. Conetainers and rose pots were watered with DI water (Table 6) for two months before plants and soil were harvested for water potential measurements (see **Water Potential** in main text for further details). Water potential measurements confirmed effects of water volume and salinity on both plants and soil, justifying use of these treatments.

## **Appendix 2 – Greenhouse Experiment**

Eight-ounce Styrofoam cups were used to hold conetainers and rose pots upright, isolating individuals, facilitating drainage, and allowing us to rotate position of individuals while maintaining a randomized layout (Fig. 4). To hold conetainers, we cut holes in the bottom of cups, inverted them, and inserted conetainers through the holes. To accommodate the wider and heavier rose pots, we stood cups upright, cutting the bottom of the cup completely away to facilitate drainage. On greenhouse tables, we blocked individuals of the same species together, fully randomizing the position of individuals within these blocks. Cups within blocks were moved two spaces horizontally and two spaces vertically every 14 days, with species blocks completing a circuit around greenhouse tables within 10 weeks.