Supplemental Information

Article title: Creek system and regional patterns of Juncus roemerianus stress and links to salt marsh loss on the Florida Gulf Coast

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Online Resource 1. Additional methodological details and mean and standard error of *Juncus roemerianus* responses and study site environmental characteristics.

Table OR1-1. Physiological parameters linked to Juncus response to stress measured, along with the justification for the variable as a measurement of Juncus stress, and the methodology for that parameter.

Parameter	Justification and Methodology
Above and belowground biomass	**Justification:** Biomass is the primary measure of productivity for macrophytes. In high salinity, resources are allocated to mediating salinity stress and directed away from productivity (Solohin et al 2020). **Methodology:** To measure *Juncus* productivity, at each marsh position plot, we harvested all *Juncus* growth (above- and belowground) in one of the three 0.25 × 0.25 m subplots. Plant biomass was sorted into above- and belowground material, thoroughly rinsed, and dried to a constant weight in a forced air oven at 70°C.
Stem density and stem height	**Justification:** Stem density and stem height are measures of productivity that may reveal patterns of growth in response to environmental conditions or stress (Solohin et al 2020). **Methodology:** At each marsh position plot, we haphazardly established three 0.25 × 0.25 m subplots to measure stem density (counted the total stems in each subplot) and stem height (measured the tallest 7 stems in each subplot).
Relative stem water content*	**Justification:** Beyond a threshold level, increases in salinity promote an increase in water content to accommodate increased vacuolar volume for ion accumulation (Gulzar et al 2003). **Methodology:** For 10 stems, relative stem water content was determined as the difference in wet stem mass and dried stem mass, stems were dried in a forced air oven at 70°C to constant weight.
Stomate density *	**Justification:** Elevated stomatal density indicates reduced transpirational efficiency resulting from salinity stress(Touchette 2006). **Methodology:** Three impressions/shoot on 3 random shoots were made at the time of stem harvest by applying a thin coat of nail polish over the leaf in a 1.5 × 4

	cm area located approximately 30 cm below the tip of the stem. The impression was removed with clear tape and placed on a microscope slide. Using an Olympus CX23 microscope (Olympus Corp, Pennsylvania, USA) stomatal density (stomates mm^{-2}) was determined on each impression from 3 fields of view at 400x (Touchette et al 2009a).
Proline	**Justification:** High levels of proline accumulation under salt stress indicate this plant defense against osmotic stress is at work (Cavalieri and Huang 1979). **Methodology:** Plant stems were flash-frozen to −60 °F with medical-grade freeze spray when harvested, stored in dry ice until returned to the lab, and stored for no more than 7 days in freezers at −80°C. Proline enzyme levels (n = 12 stems) were extracted and measured (Bates et al 1973; Ábrahám et al 2010).
Tissue ions (Ca^+, Mg^+, K^+, P^+, and Na^+)	**Justification:** *Juncus* growth is reduced when its ability to restrict salt from plant roots is insufficient to maintain ionic balance (Touchette et al 2009b), which typically occurs at levels greater than 30ppt salinity (Eleuterius 1984). Above this level, salts enter plant tissues, disrupt protein synthesis, and upset osmotic balance via Na^+ ions' replacement of other cations on membrane binding sites, thus reducing plant growth (Hu and Schmidhalter 2005). **Methodology:** Four stems were coarsely ground and analyzed for ion content via near infrared reflectance spectroscopy at the University of Florida Forage Evaluation Support Laboratory. Na^+ tissue content was also analyzed with a handheld LaquaTwin Na^+ ion meter (Horiba, Japan). Dried leaf material was ground and added (20 mg) to 2 ml centrifuge tubes with 500 µl of deionized water and placed on a shaker for 2 hours. Samples were centrifuged at 13,000 rpm for 5 minutes, and 300 µl of supernatant was placed on the Na^+ meter (Iseki et al. 2017). Lignin and crude protein levels were measured on a subset of *Juncus* stems due to costly and intensive processing for these parameters, and we found no significant trend with marsh position or creek system.

*We did not measure stem water content or transpiration directly due to methodological and equipment limitations for this field study. We acknowledge the physiological relationships between transpiration, soil water content, and temperature. Samples were collected during similar tidal cycles and ambient air temperatures to reduce environmental influences on the abovementioned collected factors.

References

Ábrahám E, Hourton-Cabassa C, Erdei L, Szabados L (2010) Methods for determination of proline in plants. In: Sunkar R (ed) Plant stress tolerance: Methods and protocols. Humana Press, Totowa, NJ, pp 317–331

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39:205–207. doi: 10.1007/BF00018060

Cavalieri AJ, Huang AHC (1979) Evaluation of proline accumulation in the adaptation of diverse species of marsh halophytes to the saline environment. American Journal of Botany 66:307–312. doi: 10.2307/2442607

Eleuterius LN (1984) Autecology of the Black Needlerush Juncus roemerianus. Gulf and Caribbean Research 7:339–350. doi: 10.18785/grr.0704.05

Gulzar S, Khan MA, Ungar IA (2003) Salt Tolerance of a Coastal Salt Marsh Grass. Communications in Soil Science and Plant Analysis 34:2595–2605. doi: 10.1081/CSS-120024787

Hu Y, Schmidhalter U (2005) Drought and salinity: A comparison of their effects on mineral nutrition of plants. Journal of Plant Nutrition and Soil Science 168:541–549. doi: 10.1002/jpln.200420516

Solohin E, Widney SE, Craft CB (2020) Declines in plant productivity drive loss of soil elevation in a tidal freshwater marsh exposed to saltwater intrusion. Ecology 101:e03148. doi: 10.1002/ecy.3148

Touchette BW (2006) Salt tolerance in a Juncus roemerianus brackish marsh: Spatial variations in plant water relations. Journal of Experimental Marine Biology and Ecology 337:1–12. doi: 10.1016/j.jembe.2006.05.011

Touchette BW, Rhodes KL, Smith GA, Poole M (2009a) Salt Spray Induces Osmotic Adjustment and Tissue Rigidity in Smooth Cordgrass, Spartina alterniflora (Loisel.). Estuaries and Coasts 32:917–925. doi: 10.1007/s12237-009-9178-4

Touchette BW, Smith GA, Rhodes KL, Poole M (2009b) Tolerance and avoidance: Two contrasting physiological responses to salt stress in mature marsh halophytes Juncus roemerianus Scheele and Spartina alterniflora Loisel. Journal of Experimental Marine Biology and Ecology 380:106–112. doi: 10.1016/j.jembe.2009.08.015

Table OR1-2. Additional methodological details for elevation measurements, water level and groundwater measurements, and soil collection and analysis.

Measurements/ Analysis	Methodology
Elevation measurements	Elevation data were compiled from pre-existing lidar data sets (for all 12 creek systems) as well as in situ Real Time Kinematic (RTK) Global Positioning System (GPS) point observations collected at field sites. We used freely available lidar data from the Continuously Updated Digital Elevation Model 1/9th arc-second resolution bathymetric-topographic tiles, available from the National Oceanic and Atmospheric Administration (https://coast.noaa.gov/dataviewer/#/). RTK elevation measurements (NAVD88) were recorded along transects extending from the creek margin to marsh platform plots for each of the 3 distances from the open-water coast (Fig. 2a). Creek and transect accessibility limitations, lost data, and base station elevation availability restricted RTK GPS data collection to 9 of the 12 marsh study sites. RTK GPS position and elevation measurements were collected at approximately 5 m intervals along the transects (manufacturer reported uncertainties of 0.008 m in the horizontal and 0.015 m in the vertical, Trimble 2017). Additional RTK GPS measurements were taken into the tidal creek channel to identify creek margin/natural levee morphology.
Water and groundwater salinity measurements	Groundwater salinity and tidal flood depth were measured using automated CTD Divers at each mid-creek marsh platform plot (D1271, van Essen Instruments, Netherlands). Groundwater sampling wells were installed using 5 cm diameter PVC pipe with a 50 cm well screen at a depth of approximately 0.75–1.0 m below the marsh surface. Each CTD Diver was suspended 5 cm above the well bottom to measure water conductivity, temperature, and pressure. Conductivity readings were converted into salinity using the Merelac package in R (version 3.6.2). Baro-Divers were installed at the top of the well casing in 2 wells (Creeks HC and MC) to collect local atmospheric pressures in the northern and southern ranges of the study area (DI800, van Essen Instruments, Netherlands) to assess groundwater depth and tidal characteristics. Water column height above/below each diver was calculated by the difference between pressure measured by the CTD Diver and the barometric pressure measured by the nearest Baro-Diver. Tidal flood depth was determined from the difference in surface soil elevation and the calculated water column height. Tidal statistics (average duration of inundation and dry time, average inundation height, and observed inundated tidal cycles) were calculated using the Tides package in R (version 3.6.2).

Table OR1-3. Summary of tide characteristics in each salt marsh creek system at the groundwater monitoring wells. Creeks JC and HC are not listed due to sensor failure. Creek abbreviations are identified in Table 1 of the main manuscript.

Creek System	Average water depth (cm)	High tide water height (cm)	High tide duration per cycle (hr)	Max inundation time (hr)	Low tide water height (cm)	Low tide duration per cycle (hr)	Max dry time (hr)
BC	-4.03	18.5	4.10	32.0	4.71	10.3	199
BCK	8.24	23.0	9.52	1640	5.27	1.58	97.5
CC	8.38	21.2	10.0	1050	8.76	2.32	181
EP	2.21	25.9	5.07	83.0	5.85	9.27	110
FBC	3.5	23.4	5.25	528	2.34	5.60	110
HI	7.41	29.2	7.97	1250	2.90	4.20	95.0
MC	20.9	42.7	7.02	1364	12.9	0.70	86.0
RC	9.63	19.4	4.52	1270	6.14	0.63	70.0
RI	-2.63	19.0	4.23	182	2.23	11.7	198
SC	6.19	18.7	6.08	1660	3.33	1.63	94.5
Average	5.98	24.1	6.37	906	5.45	4.80	124

Table OR1-4. Means ± standard error of *Juncus* productivity parameters for each creek system. Creek abbreviations are identified in Table 1 of the main manuscript.

Creek	Stem density (stems/m	Aboveground biomass (g)	Belowground biomass (g)	A:B biomass ratio	Stem height (cm)	Stem length:mass ratio
BC	575 ± 80	585 ± 52	449 ± 52	1.38 ± 0.19	130 ±	77.6 ± 5.9
BCK	778 ± 79	798 ± 122	396 ± 38	2.05 ± 0.27	142 ±	74.7 ± 6.3
CC	454 ± 62	568 ± 50	716 ± 127	0.99 ± 0.23	147 ±	70.3 ± 5.5
EP	454 ± 50	695 ± 52	561 ± 49	1.30 ± 0.17	155 ±	60.4 ± 4.2
FBC	288 ± 15	523 ± 115	588 ± 114	1.22 ± 0.48	129 ±	72.9 ± 6.1
HC	467 ± 55	308 ± 31	292 ± 70	2.24 ± 1.22	111 ±	73.6 ± 6.0
HI	436 ±26	622 ± 125	540 ± 109	1.35 ± 0.25	148 ±	63.6 ± 5.1
JC	486 ± 50	758 ± 146	420 ± 62	1.90 ± 0.35	165 ±	58.1 ± 4.3
MC	259 ± 32	636 ± 58	665 ± 131	1.24 ± 0.31	182 ±	54.7 ± 4.6
RC	500 ± 59	736 ± 95	551 ± 53	1.41 ± 0.25	141 ±	72.8 ± 4.6
RI	658 ± 76	666 ± 63	285 ± 50	3.13 ± 1.10	137 ±	65.0 ± 2.7
SC	637 ± 52	789 ± 63	323 ± 21	2.48 ± 0.24	152 ±	66.9 ± 6.1
Overall mean	499 ± 53	640 ±	482 ±	1.72 ±	145 ±	67.5 ±

Table OR1-5. Means ± standard error of *Juncus* physiological response parameters for each creek system. Refer to manuscript Table 1 for creek location information.

Creek	Proline (µg/gFW)	Stem water content (%)	Stomate density (stomates/mm
BC	18.8 ±	55.6 ±	122 ±
BCK	42.3 ±	58.7 ±	117 ±
CC	33.9 ±	56.7 ±	139 ±
EP	3.04 ±	58.4 ±	127 ±
FBC	13.0 ±	58.2 ±	135 ±
HC	58.1 ±	56.7 ±	127 ±
HI	2.05 ±	59.0 ±	123 ±
JC	8.63 ±	57.8 ±	134 ±
MC	3.30 ±	59.7 ±	138 ±
RC	38.0 ±	57.0 ±	126 ±
RI	34.9 ±	56.3 ±	115 ±
SC	32.4 ±	56.9 ±	115 ±
Overall mean	24.0 ±	57.6 ±	127 ±

Table OR1-6. Means ± standard error of *Juncus* physiological response parameters for each creek system. Refer to manuscript Table 1 for creek location information.

Creek	Na^+ (ppm)	K^+ (%)	K:Na ratio	P^+ (%)	Ca^+ (%)	Mg^+ (%)
BC	25.7 ±	0.728 ±	0.0281 ±	0.203 ±	0.403 ±	0.231 ±
BCK	31.8 ±	0.341 ±	0.0115 ±	0.214 ±	0.857 ±	0.154 ±
CC	26.9 ±	0.971 ±	0.0362 ±	0.194 ±	0.347 ±	0.173 ±
EP	20.2 ±	0.683 ±	0.0359 ±	0.189 ±	0.390 ±	0.138 ±
FBC	30.6 ±	0.954 ±	0.0327 ±	0.195 ±	0.298 ±	0.142 ±
HC	31.3 ±	0.718 ±	0.0228 ±	0.187 ±	0.415 ±	0.166 ±
HI	25.5 ±	0.791 ±	0.0315 ±	0.184 ±	0.305 ±	0.157 ±
JC	28.7 ±	0.950 ±	0.0338 ±	0.210 ±	0.325 ±	0.218 ±
MC	19.4 ±	0.306 ±	0.0169 ±	0.183 ±	0.888 ±	0.123 ±
RC	29.5 ±	0.337 ±	0.0120 ±	0.223 ±	0.599 ±	0.136 ±
RI	25.4 ±	0.819 ±	0.0326 ±	0.195 ±	0.317 ±	0.192 ±
SC	22.3 ±	0.746 ±	0.0341 ±	0.184 ±	0.315 ±	0.137 ±
Overall mean	26.4 ±	0.695 ±	0.027 ±	0.197 ±	0.455 ±	0.164 ±

Table OR1-7. Means ± standard error of *Juncus* productivity parameters for three spatial factors: distance from coast, distance from creek bank, and the interaction between each.

	Stem density (stems/m^2)	Aboveground biomass (g)	Belowground biomass (g)	A:B biomass ratio	Stem height (cm)	Stem length:mass ratio
Distance from coast						
Coast	465 ± 24.5	652 ± 51.0	524 ± 41.3	1.43 ± 0.16	146.5 ± 1.4	60.6 ± 1.0
Mid-creek	512 ± 27.6	568 ± 47.7	490 ± 45.0	1.43 ± 0.14	142.2 ± 1.0	74.3 ± 1.2
Inland	521 ± 25.7	629 ± 45.4	433 ± 53.3	2.31 ± 0.41	145.1 ± 1.1	67.5 ± 1.1
Distance from creek bank						
Creek bank	511 ± 22.3	637 ± 41.5	447 ± 38.1	2.05 ± 0.29	145.8 ± 1.0	64.0 ± 0.8
Marsh platform	487 ± 20.6	596 ± 37.0	517 ± 37.9	1.40 ± 0.11	143.4 ± 1.0	71.2 ± 1.0
Distance from coast x Distance from creek bank						
Coastal-creek bank	522 ± 40.4	649 ± 75.1	524 ± 52.0	1.44 ± 0.26	144.1 ± 1.8	58.2 ± 1.2
Coastal-marsh platform	409 ± 28.6	655 ± 72.1	524 ± 66.5	1.42 ± 0.21	148.9 ± 2.2	62.9 ± 1.5
Mid-creek-creek bank	475 ± 39.0	595 ± 76.3	423 ± 56.3	1.66 ± 0.19	145.0 ± 1.4	69.1 ± 1.3
Mid-creek-marsh platform	549 ± 36.7	542 ± 59.6	557 ± 67.1	1.21 ± 0.19	139.5 ± 1.2	79.4 ± 1.8
Inland-creek bank	537 ± 36.8	668 ± 68.2	394 ± 84.5	3.06 ± 0.76	148.3 ± 1.7	64.1 ± 1.5
Inland-marsh platform	505 ± 36.1	591 ± 60.7	471 ± 66.7	1.56 ± 0.19	141.9 ± 1.5	71.2 ± 1.5

Table OR1-8. Means ± standard error of *Juncus* physiological parameters for three spatial factors: distance from coast, distance from creek bank, and the interaction between each.

	Proline (µg/gFW)	Stem water content (%)	Stomate density (stomates/mm^2)
Distance from coast			
Coast	35.7 ± 3.2	57.9 ± 0.2	128 ± 3.8
Mid-creek	22.3 ± 2.0	57.4 ± 0.4	125 ± 4.0
Inland	14.8 ± 1.5	57.3 ± 0.3	127 ± 4.1
Distance from creek bank			
Creek bank	28.1 ± 2.1	58.1 ± 0.3	123 ± 3.0
Marsh platform	20.4 ± 1.8	57.0 ± 0.2	131 ± 3.4
Distance from coast x Distance from creek bank			
Coastal-creek bank	37.1 ± 4.4	58.2 ± 0.3	121 ± 5.0
Coastal-marsh platform	34.3 ± 4.7	57.7 ± 0.4	135 ± 5.7
Mid-creek-creek bank	29.2 ± 3.5	58.4 ± 0.7	125 ± 4.8
Mid-creek-marsh platform	15.5 ± 1.9	56.6 ± 0.4	124 ± 6.5
Inland-creek bank	18.2 ± 2.5	57.7 ± 0.4	122 ± 5.6
Inland-marsh platform	11.3 ± 1.3	56.8 ± 0.3	133 ± 5.6

Table OR1-9. Means ± standard error of *Juncus* tissue ion levels for three spatial factors: distance from coast, distance from creek bank, and the interaction between each.

	Na^{+} (ppm)	K^{+} (%)	K:Na ratio	P^{+} (%)	Ca^{+} (%)	Mg^{+} (%)
Distance from coast						
Coast	266 ± 7.86	0.715 ± 0.034	0.028 ± 0.0013	0.200 ± 0.0025	0.476 ± 0.031	0.169 ± 0.0055
Mid-creek	272 ± 8.10	0.708 ± 0.035	0.026 ± 0.0012	0.195 ± 0.0026	0.448 ± 0.021	0.164 ± 0.0045
Inland	255 ± 8.59	0.663 ± 0.035	0.028 ± 0.0016	0.196 ± 0.0023	0.440 ± 0.023	0.158 ± 0.0056
Distance from creek bank						
Creek bank	280 ± 6.92	0.759 ± 0.029	0.028 ± 0.0012	0.199 ± 0.0017	0.444 ± 0.019	0.163 ± 0.0043
Marsh platform	248 ± 6.20	0.632 ± 0.027	0.026 ± 0.0011	0.194 ± 0.0023	0.465 ± 0.022	0.164 ± 0.0042
Distance from coast x Distance from creek bank						
Coastal-creek bank	279 ± 12.2	0.708 ± 0.051	0.026 ± 0.0017	0.200 ± 0.0031	0.489 ± 0.041	0.163 ± 0.0081
Coastal-marsh platform	252 ± 9.63	0.568 ± 0.048	0.030 ± 0.0012	0.199 ± 0.0040	0.464 ± 0.046	0.175 ± 0.0071
Mid-creek-creek bank	292 ± 11.3	0.721 ± 0.046	0.031 ± 0.0026	0.198 ± 0.0030	0.421 ± 0.034	0.162 ± 0.0076
Mid-creek-marsh platform	252 ± 11.0	0.811 ± 0.050	0.024 ± 0.0019	0.194 ± 0.0036	0.459 ± 0.031	0.155 ± 0.0082
Inland-creek bank	270 ± 12.4	0.756 ± 0.048	0.028 ± 0.0016	0.200 ± 0.0030	0.423 ± 0.024	0.165 ± 0.0068
Inland-marsh platform	241 ± 11.6	0.606 ± 0.044	0.025 ± 0.0017	0.189 ± 0.0042	0.473 ± 0.034	0.163 ± 0.0060