Appendix 9.E. Response of *Vallisneria americana* following repeated exposure to different salinity regimes

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Introduction

Vallisneria americana performs many valued functions in the St. Johns River, including serving as refuge for juvenile fish; providing spawning habitat for fish and invertebrates; anchoring sediment; ameliorating phytoplankton blooms by competing for nutrients; and feeding manatees, turtles, and waterfowl. This species of submerged aquatic vegetation tolerates moderate levels of salinity, which allows it to grow and reproduce through much of the river's lower basin. Previous studies primarily documented the responses of *V. americana* following one-time exposures to different salinities for varying durations (e.g., Kraemer et al. 1999; Doering et al. 2001; French and Moore 2003; Frazer et al. 2006; Jarvis and Moore 2008; Dobberfuhl et al. 2009; Boustany et al. 2010). In combination, these data generate models predicting stress, mortality, and declining germination rates (Figures 1 and 2).

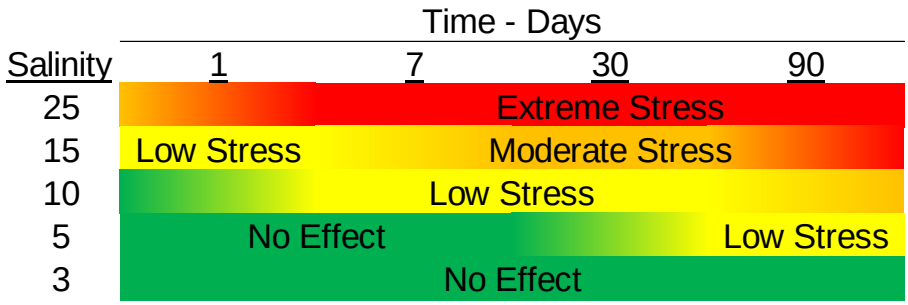

Figure 1. Impacts of salinity on *Vallisneria americana* (figure from Dobberfuhl et al. 2009).

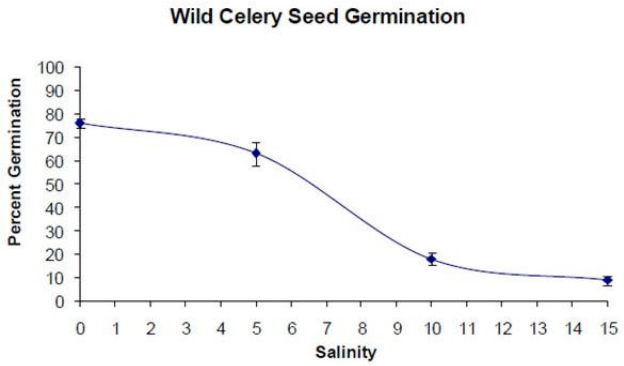

Figure 2. Impacts of salinity on germination of *Vallisneria americana* seedlings (data from Jarvis and Moore 2008, figure from Dobberfuhl et al. 2009).

Predictions from previous studies did not address impacts of repeated exposures to given salinities, especially non-lethal salinities predicted to cause stress. *Vallisneria americana* in the lower St. Johns River encounters potentially stressful salinities (Figure 3), and such exposures have contributed to loss of plants (Dobberfuhl et al. 2009; Sagan 2009). In many cases, *V. americana* recovered when salinities decreased following one-time increases, but the limits to its capacity to recover from repeated stresses remain unknown. Increases in salinity may become more frequent with water withdrawals, i.e., "return intervals" may become shorter; therefore, an understanding of how *V. americana* responds to repeated salinity increases will promote sustainable use of alternative water supplies. This manipulative field experiment assessed the responses of *V. americana* to salinity stresses with different, experimentally imposed "return intervals."

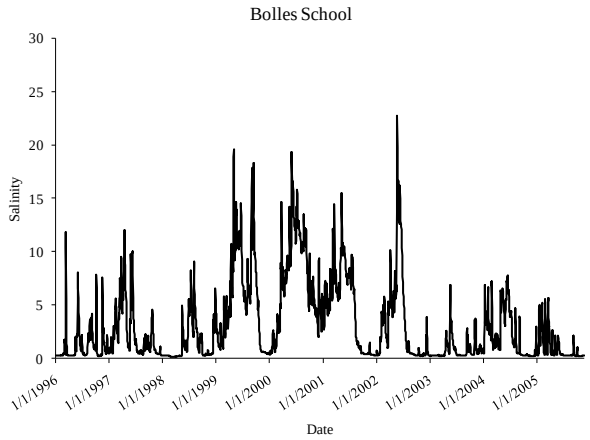

Figure 3. Salinities measured within a bed of submerged aquatic vegetation.

Materials and Methods

Study sites

The St. Johns River in northeast Florida originates in extensive wetlands before flowing northward for approximately 500 km and discharging into the Atlantic Ocean. Wetlands along the river supply chromophoric dissolved organic matter that creates highly colored water (typically 100–500 platinum cobalt units). The river's lower basin, which was the location for this study, comprises its final 177 km running from the confluence of the St. Johns and Ocklawaha rivers to the Atlantic Ocean. The lower basin is essentially estuarine, but the location of the halocline varies. In fact, the bed of the river remains below sea level for 400 of its 500 km length, which makes the lower basin subject to tidal activity and occasional reverse flow events resulting from low discharge rates during droughts or winds associated with persistent weather fronts. Extreme intrusions can introduce saline water 268 km upstream to near Sanford, Florida.

Experiments took place at four sites representing a range of salinity regimes (Table 1 and Figure 4). The first experiment utilized the Murphy Island, Bolles School and Jacksonville University sites. In the second experiment, Alpine Groves replaced the Jacksonville University site because it provided salinities intermediate to those at Murphy Island and Bolles School.

Table 1. Historical salinity regimes at study sites as derived from modeling based on a 10-year period.

Parameter	Jacksonville University	Bolles School	Alpine Groves	Murphy Island
Minimum	0.3	0.2	0.2	0.2
Maximum	33.0	23.4	16.4	0.8
Mean	17.6	3.2	2.0	0.4
Median	17.7	1.4	0.5	0.4

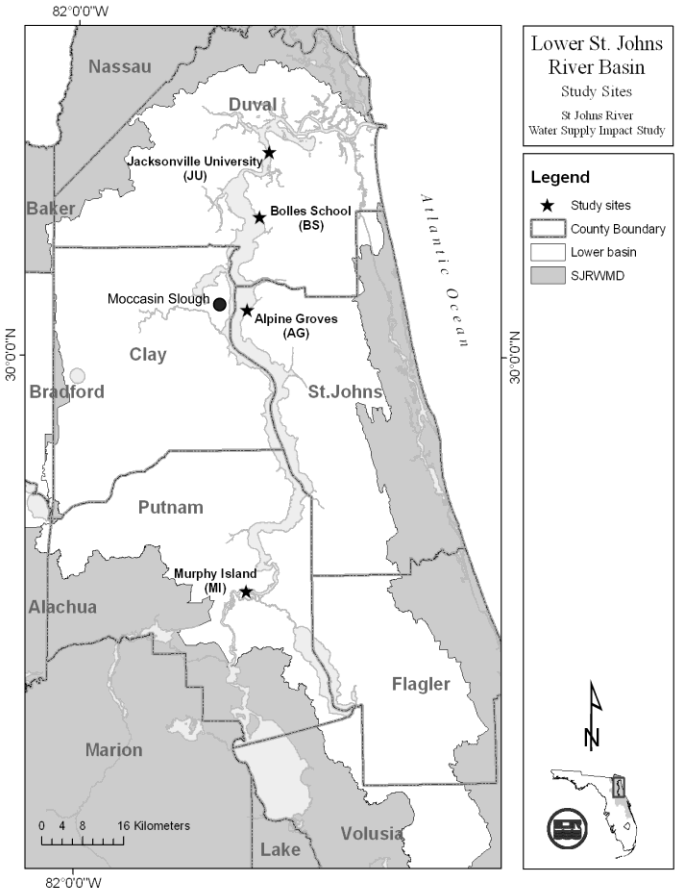

Figure 4. Map of the lower St. Johns River basin showing study sites (stars) and the Moccasin Slough collection site for *Vallisneria americana* (circle)

Experimental design

Vallisneria americana transplants were taken from a site adjacent to the Murphy Island experimental site and at the northernmost site with plants at the time of the experiments. This site, Moccasin Slough, is located along the western shore of the river near the Alpine Groves experimental site. These sites provided plants acclimated to different salinity regimes. Cores containing sediment and *V. americana* ramets were placed in plastic pots with 15-cm diameters and a porous fabric lining to limit loss of sediment. Pots held in bins were anchored to the sediment at an average depth of 0.5 m, each bin held four pots, and bins were paired to create sets of eight replicates (Figure 5). Pots and bins had holes that promoted equilibration between porewater and the water column. At each site, groups of bins formed two stations separated by 3–5 m. Plants in pots equilibrated for 5–7 d before experimental manipulations.

Both experiments required 320 pots distributed among four temporal cycles and five combinations of site of origin and site of exposure (Table 2; Figure 5). Experimental treatments consisted of transporting pots to sites typified by higher salinities for the appropriate interval, with each exposure followed by an equal recovery period at the appropriate site of origin. Paired exposure and recovery periods (cycles) varied between 6 and 42 days. Pots that remained at their sites of origin and received all scheduled handling served as controls for the effects of transportation, and pots exposed to higher salinities for the duration of each experiment served as controls for handling and transportation. During transport, submerging pots in bins of water minimized such handling stress.

Table 2. Design of transplant experiments, with the 16 pots in each grouping split evenly between two stations at each site. NA = not applicable

Site of exposure	Number of pots in Experiment 1				Number of pots in Experiment 2			
	Cycle (d)	Site of origin		Total	Cycle (wk)	Site of origin		Total
		Murphy Island	Moccasin Slough			Murphy Island	Moccasin Slough	
Jacksonville University	3–5	16	16	32	NA	NA	NA	NA
	6–9	16	16	32	NA	NA	NA	NA
	10–12	16	16	32	NA	NA	NA	NA
	Duration	16	16	32	NA	NA	NA	NA
Bolles School	3–5	16	16	32	1	16	16	32
	6–9	16	16	32	2	16	16	32
	10–12	16	16	32	3	16	16	32
	Duration	16	16	32	Duration	16	16	32
Alpine Groves	NA	NA	NA	NA	1	16	16	32
	NA	NA	NA	NA	2	16	16	32
	NA	NA	NA	NA	3	16	16	32
	NA	NA	NA	NA	Duration	16	16	32
Murphy Island	3–5	16	NA	16	1	16	NA	16
	6–9	16	NA	16	2	16	NA	16
	10–12	16	NA	16	3	16	NA	16
	Duration	16	NA	16	Duration	16	NA	16
Total		192	128	320		192	128	320

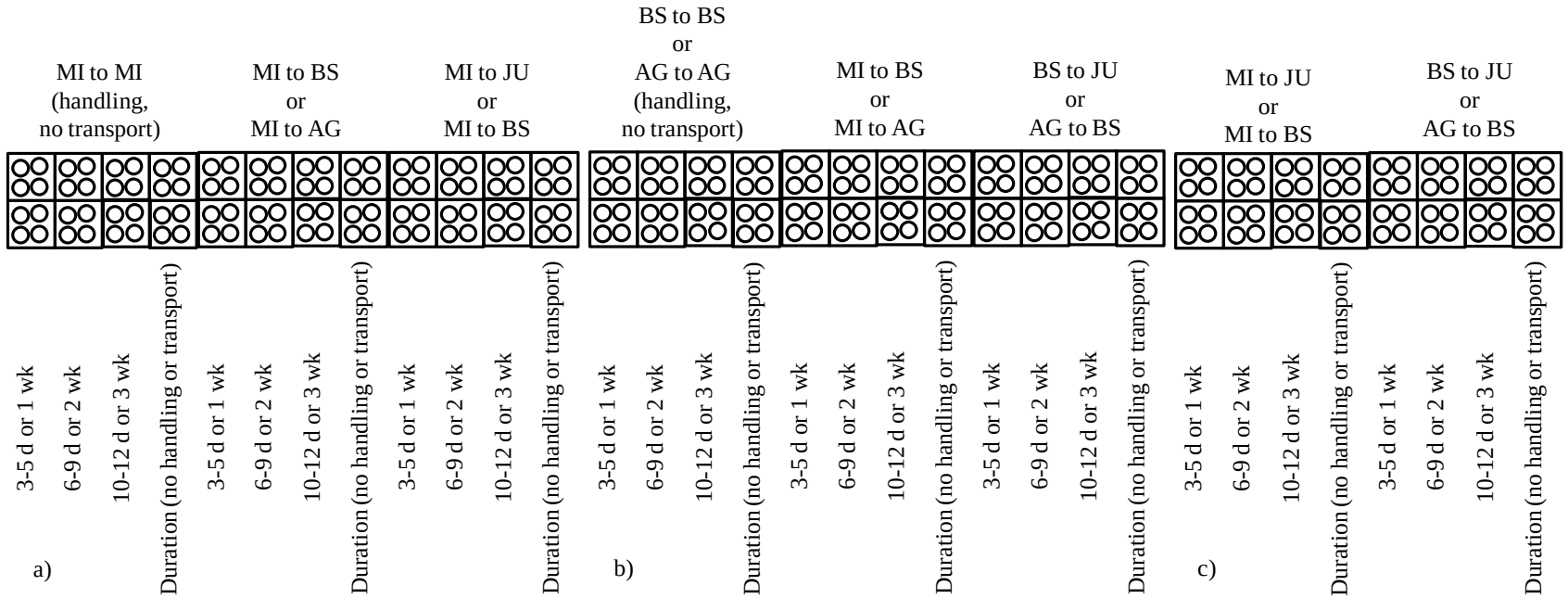

Figure 5. Diagram showing the arrangement of pots at one of two stations at a) Murphy Island in both experiments, b) Bolles School in the first experiment and Alpine Groves in the second experiment, and c) Jacksonville University in the first experiment and Bolles School in the second experiment.

Field conditions

In situ sampling and analysis of grab samples characterized the key drivers of stress for *Vallisneria americana*. Submersible datasondes (YSI 600 series) and paired underwater and surface light sensors (Li-Cor 2π sensors and Li-Cor 1000 or 1400 light meters) provided high frequency, *in situ* measurements of salinity, water temperature and photosynthetically active radiation. In addition, once each week, water depth, Secchi depth, salinity, water temperature and pH (YSI 600 series datasonde with 650 series multiparameter display system) were recorded at each site, and water samples were taken for determination of concentrations of various nitrogen and phosphorus compounds, organic carbon, total suspended solids, dissolved oxygen, color, and chlorophyll.

Viability of Vallisneria americana

Counts of ramets (shoots), live blades and dead blades made at the start of the experiment, at the end of each period of exposure and recovery, or at selected times documented the condition of transplants during both experiments. Records of reproductive activity, visual estimates of epiphyte coverage (0–25%, 26–50%, 51–75% and 76–100%), and notes on general condition yielded qualitative data. During the 1–3 h required for counting and transporting, pots remained in bins of water collected at the experimental sites.

During the second experiment, analyses of total soluble protein, total antioxidant capacity and catalase activity yielded measures of sub-lethal stress. Samples comprised single blades collected from two pots (one from each station for each combination of temporal cycle, site of origin and site of treatment) at the beginning of each round of counting. Handling of the resulting duplicate samples involved sealing them in plastic bags, storing them on ice and freezing them (-4°C) prior to transporting them to the University of North Florida.

At the University of North Florida, 50–200 mg of tissue were homogenized using a FastPrep-24 bead homogenizing system (MP Biomedicals, Solon, OH, USA). The homogenate was created in 500 µl of ice cold tris-hydrochloride buffer, with a pH of 8.0 and addition of 1% polyvinylpyrrolidone (weight to volume) and 0.1% protease inhibitor cocktail (volume to volume; Sigma, St. Louis, MO, USA). The homogenate was centrifuged for 10 min at 16,000× gravity, and the resulting supernatant was collected and assayed for total soluble protein content using the Pierce® BCA Protein Assay Kit (Rockford, IL, USA). The concentration of soluble protein, as a function of fresh mass, was converted to percent soluble protein. The total antioxidant capacity of extracted tissue was quantified using the method described by Re et al. (1999). Amplex Red Catalase assays, employed as per the manufacturer's directions (Invitrogen, Eugene, OR, USA), yielded catalase activity. Total antioxidant capacity and catalase activity were standardized to percentages of soluble protein.

Statistical analyses

Data (i.e., measures of water quality parameters, counts of live blades, counts of ramets, and standardized measures of total antioxidant capacity and catalase activity) were used in analyses of variance (ANOVAs). As appropriate, ANOVAs involved sites as levels of a fixed factor, time during experiments as levels of a fixed factor, combinations of sites of origin and treatment as levels of a fixed factor, stations nested in sites, and pots nested in sites and stations to address the repeated measures nature of the experiment. Before ANOVAs, data were tested for homoscedasticity with Cochran's tests and normality with Anderson–Darling tests. Data were transformed to meet these assumptions, and significant results were interpreted conservatively if the assumptions were not met. For water quality parameters, one-half of the detection limit replaced values that were below detection limits (Antweiler and Taylor 2008). In cases where logistics and unforeseen circumstances obviated plans to collect data, means of appropriate data replaced missing values and degrees of freedom were reduced.

Results

Field conditions

A series of ANOVAs indicated differences in water quality parameters across sites (Tables 3 and 4). The relationship between Secchi depth and total depth along with color values and concentrations of chlorophyll *a* and total suspended solids characterized water clarity, which determined the light regime at the sites. Concentrations of various nitrogen and phosphorus species documented variations in the supply of nutrients. In combination with temperature, light and nutrients represented factors that could have confounded changes due to exposure to differing salinities.

Table 3. Results of tests of assumptions and analyses of variance for water quality parameters measured during the first experiment.

Parameter	Cochran's	Anderson–Darling	Source	DF	SS	MS	*F*	*p*
Secchi depth ÷ Total depth	$p > 0.05$	$p > 0.05$	Site	2	0.315	0.157	1.66	0.231
			Error	12	1.137	0.095		
Color	$p > 0.05$	$p < 0.005$	Site	2	0.039	0.020	0.06	0.945
(cobalt platinum units)			Error	12	4.085	0.340		
Total suspended solids	$p > 0.05$	$p > 0.05$	Site	2	1.293	0.647	5.72	0.018
(mg L^{-1})			Error	12	1.356	0.113		
Corrected chlorophyll *a*	$p > 0.05$	$p > 0.05$	Site	2	2.682	1.341	101.21	< 0.001
(mg m^{-3})			Error	12	0.159	0.013		
Total Kjeldahl nitrogen	$p < 0.01$	$p < 0.005$	Site	2	0.110	0.055	2.58	0.117
(mg L^{-1})			Error	12	0.255	0.021		
Nitrate + Nitrite	$p < 0.01$	$p > 0.01$	Site	2	1.818	0.909	38.28	< 0.001
(mg L^{-1})			Error	12	0.285	0.0238		
Ammonium	$p < 0.01$	$p > 0.05$	Site	2	0.734	0.367	3.07	0.084
(mg L^{-1})			Error	12	1.436	0.120		
Total phosphorus	$p > 0.05$	$p < 0.01$	Site	2	0.386	0.193	31.80	< 0.001
(mg L^{-1})			Error	12	0.0728	0.006		
Phosphate	$p > 0.01$	$p > 0.05$	Site	2	0.972	0.486	46.68	< 0.001
(mg L^{-1})			Error	12	0.125	0.010		
Temperature	$p > 0.05$	$p > 0.01$	Site	2	0.0002	0.0001	0.07	0.935
(°C)			Error	12	0.0252	0.0021		
Salinity	$p > 0.05$	$p > 0.05$	Site	2	4.877	2.438	93.16	< 0.001
			Error	12	0.314	0.026		

Table 4. Results of tests of assumptions and analyses of variance for water quality parameters measured during the second experiment.

Parameter	Cochran's	Anderson–Darling	Source	DF	SS	MS	*F*	*p*
Secchi depth ÷ Total depth	$p > 0.05$	$p < 0.01$	Site	2	0.217	0.108	1.45	0.245
			Error	53	3.970	0.075		
Color	$p > 0.05$	$p > 0.05$	Site	2	0.026	0.013	2.61	0.083
			Error	53	0.264	0.005		
Total suspended solids	$p > 0.05$	$p > 0.05$	Site	2	0.175	0.088	1.68	0.195
			Error	53	2.756	0.052		
Corrected chlorophyll *a*	$p > 0.05$	$p > 0.05$	Site	2	3.506	1.753	32.07	< 0.001
			Error	53	2.897	0.055		
Total Kjeldahl nitrogen	$p > 0.05$	$p > 0.05$	Site	2	0.0135	0.007	5.06	0.010
			Error	53	0.0705	0.001		
Nitrate + Nitrite	$p > 0.05$	$p < 0.01$	Site	2	1.997	0.999	4.66	0.014
			Error	53	11.368	0.214		
Ammonium	$p > 0.05$	$p < 0.01$	Site	2	0.033	0.017	0.35	0.705
			Error	53	2.510	0.047		
Total phosphorus	$p > 0.05$	$p > 0.01$	Site	2	3.269	1.634	116.59	< 0.001
			Error	53	0.743	0.014		
Phosphate	$p > 0.05$	$p > 0.05$	Site	2	7.949	3.9743	100.60	< 0.001
			Error	53	2.094	0.040		
Temperature	$p > 0.05$	$p < 0.01$	Site	2	0.003	0.002	1.50	0.233
			Error	53	0.058	0.001		
Salinity	$p < 0.01$	$p > 0.05$	Site	2	8.455	4.227	61.99	< 0.001
			Error	53	3.614	0.068		

Light attenuating substances exhibited varying patterns across the sites in both experiments (Tables 3, 4, 5 and 6). In the first experiment, higher total suspended solids concentrations were recorded at Jacksonville University, whereas, chlorophyll *a* concentrations remained higher at Murphy Island in both experiments. Color did not vary significantly among sites in either experiment and neither did an overall measure of water clarity created by dividing the Secchi depth by the depth of water at the relevant site. Thus, the key influences on light climate at the sites differed, but the overall climate remained similar, as confirmed by photosynthetically active radiation sensors deployed successfully at Murphy Island and Bolles School during the second experiment. On average, 35% and 9% of incident light reached the *Vallisneria americana* at Murphy Island and Bolles School experimental sites, respectively. Therefore, plants should not have been subject to significant light stress.

During both experiments, mean concentrations of macronutrients, i.e., nitrogen and phosphorus species tended to be higher at Jacksonville University and Bolles School, the downstream sites (Tables 3, 4, 5 and 6). In contrast, mean total Kjeldahl nitrogen concentrations were highest at Murphy Island during the second experiment. Overall, the concentrations of nutrients were within normal ranges for the St. Johns River, and therefore, they should not have adversely affected the viability of *Vallisneria americana*.

Temperatures did not vary significantly among the sites in either experiment (Tables 3, 4, 5 and 6; Figure 6). As expected, temperatures during the first experiment, November and December 2009, were lower than temperatures during the second experiment, June to October 2010. The consistency of temperatures across sites should have obviated any differential temperature stress on *Vallisneria americana*.

The experiments subjected *Vallisneria americana* plants to differing salinities, and mean salinities differed significantly among sites in both experiments (Tables 3, 4, 5 and 6; Figure 7). As expected, the downstream sites had higher salinities. During the first experiment, plants moved to Jacksonville University experienced salinity to 18.8, plants moved to Bolles School experienced salinity to 8.8, and plants at Murphy Island experienced a maximum salinity of 0.5. Plants at Murphy Island and Bolles School experienced the same maximum salinities during the second experiment, whereas plants at Alpine Groves experienced salinity to 5.0 in the latter half of the experiment. Thus, experimental manipulations created the desired variations in exposure to salinity, with auxiliary effects from varying light climates and temperatures minimized.

Table 5. Summary statistics for water quality parameters during the first experiment.

Parameter	Site	Mean	95% confidence limits	
			Lower	Upper
Secchi depth/Total depth	Murphy Island	0.51	0.12	0.45
	Bolles School	0.78	0.67	0.88
	Jacksonville University	0.48	0.15	0.57
Color	Murphy Island	88	42	187
(cpu)	Bolles School	93	43	202
	Jacksonville University	71	35	145
Total suspended solids	Murphy Island	10.6	6.9	16.4
(mg L^{-1})	Bolles School	9.8	8.4	11.3
	Jacksonville University	42.6	23.7	76.7
Corrected chlorophyll a	Murphy Island	17.5	15.3	20.0
(mg m^{-3})	Bolles School	2.4	2.1	2.7
	Jacksonville University	2.1	1.7	2.5
Total Kjeldahl nitrogen	Murphy Island	0.849	0.796	0.905
(mg L^{-1})	Bolles School	0.907	0.878	0.938
	Jacksonville University	0.580	0.423	0.795
Nitrate + Nitrite	Murphy Island	0.067	0.048	0.094
(mg L^{-1})	Bolles School	0.392	0.382	0.403
	Jacksonville University	0.344	0.318	0.372
Ammonium	Murphy Island	0.033	0.030	0.036
(mg L^{-1})	Bolles School	0.019	0.016	0.022
	Jacksonville University	0.065	0.031	0.138
Total phosphorus	Murphy Island	0.049	0.044	0.053
(mg L^{-1})	Bolles School	0.100	0.098	0.102
	Jacksonville University	0.112	0.097	0.129
Phosphate	Murphy Island	0.023	0.817	1.224
(mg L^{-1})	Bolles School	0.077	0.940	1.064
	Jacksonville University	0.080	0.924	1.082
Temperature	Murphy Island	19.4	18.6	20.2
(°C)	Bolles School	18.9	17.6	20.4
	Jacksonville University	19.2	18.1	20.2
Salinity	Murphy Island	0.4	0.4	0.5
	Bolles School	3.6	2.8	4.7
	Jacksonville University	10.5	8.2	13.5

Table 6. Summary statistics for water quality parameters during the second experiment.

Parameter	Site	Mean	95% confidence limits	
			Lower	Upper
Secchi depth/Total depth	Murphy Island	0.97	0.91	1.00
	Alpine Groves	0.91	0.81	0.97
	Bolles School	0.97	0.91	1.00
Color	Murphy Island	60	57	65
(cpu)	Alpine Groves	57	53	61
	Bolles School	53	48	59
Total suspended solids	Murphy Island	17.1	14.2	20.5
(mg L^{-1})	Alpine Groves	13.3	10.4	16.9
	Bolles School	12.8	9.2	17.8
Corrected chlorophyll a	Murphy Island	46.2	36.1	59.1
(mg m^{-3})	Alpine Groves	21.7	16.1	29.1
	Bolles School	11.2	8.8	14.3
Total Kjeldahl nitrogen	Murphy Island	0.720	0.684	0.758
(mg L^{-1})	Alpine Groves	0.718	0.694	0.743
	Bolles School	0.666	0.644	0.690
Nitrate + Nitrite	Murphy Island	0.006	0.004	0.011
(mg L^{-1})	Alpine Groves	0.004	0.003	0.008
	Bolles School	0.013	0.008	0.020
Ammonium	Murphy Island	0.031	0.023	0.040
(mg L^{-1})	Alpine Groves	0.027	0.022	0.032
	Bolles School	0.028	0.021	0.037
Total phosphorus	Murphy Island	0.014	0.012	0.016
(mg L^{-1})	Alpine Groves	0.024	0.021	0.028
	Bolles School	0.053	0.048	0.059
Phosphate	Murphy Island	0.004	0.003	0.005
(mg L^{-1})	Alpine Groves	0.009	0.007	0.011
	Bolles School	0.032	0.030	0.034
Temperature	Murphy Island	30.0	28.7	31.2
(°C)	Alpine Groves	28.8	27.8	29.8
	Bolles School	29.9	28.8	31.0
Salinity	Murphy Island	0.5	0.4	0.5
	Alpine Groves	1.3	0.9	1.9
	Bolles School	4.2	3.1	5.8

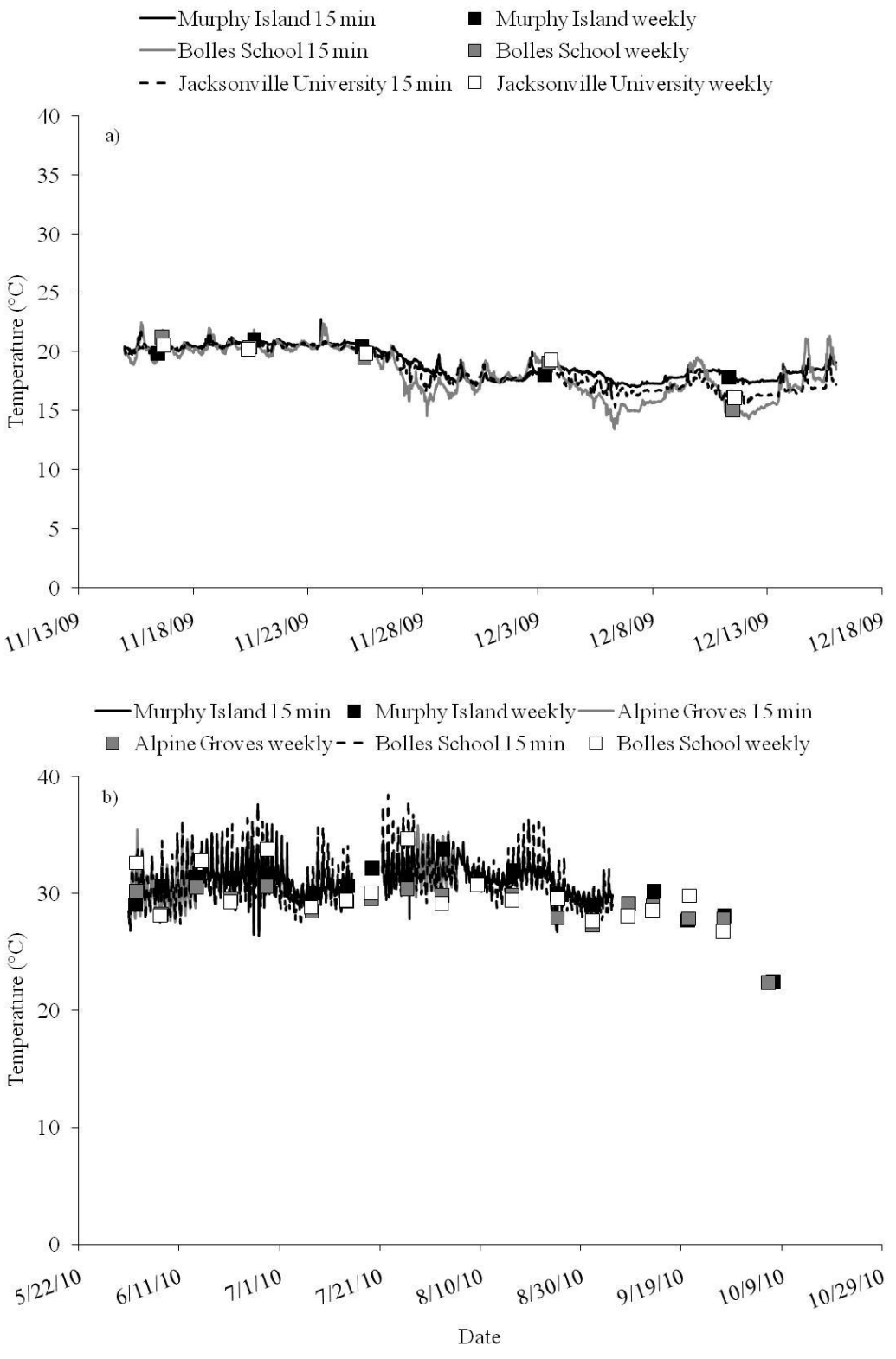

Figure 6. Water temperatures from weekly and 15-minute sampling for a) the first experiment and b) the second experiment.

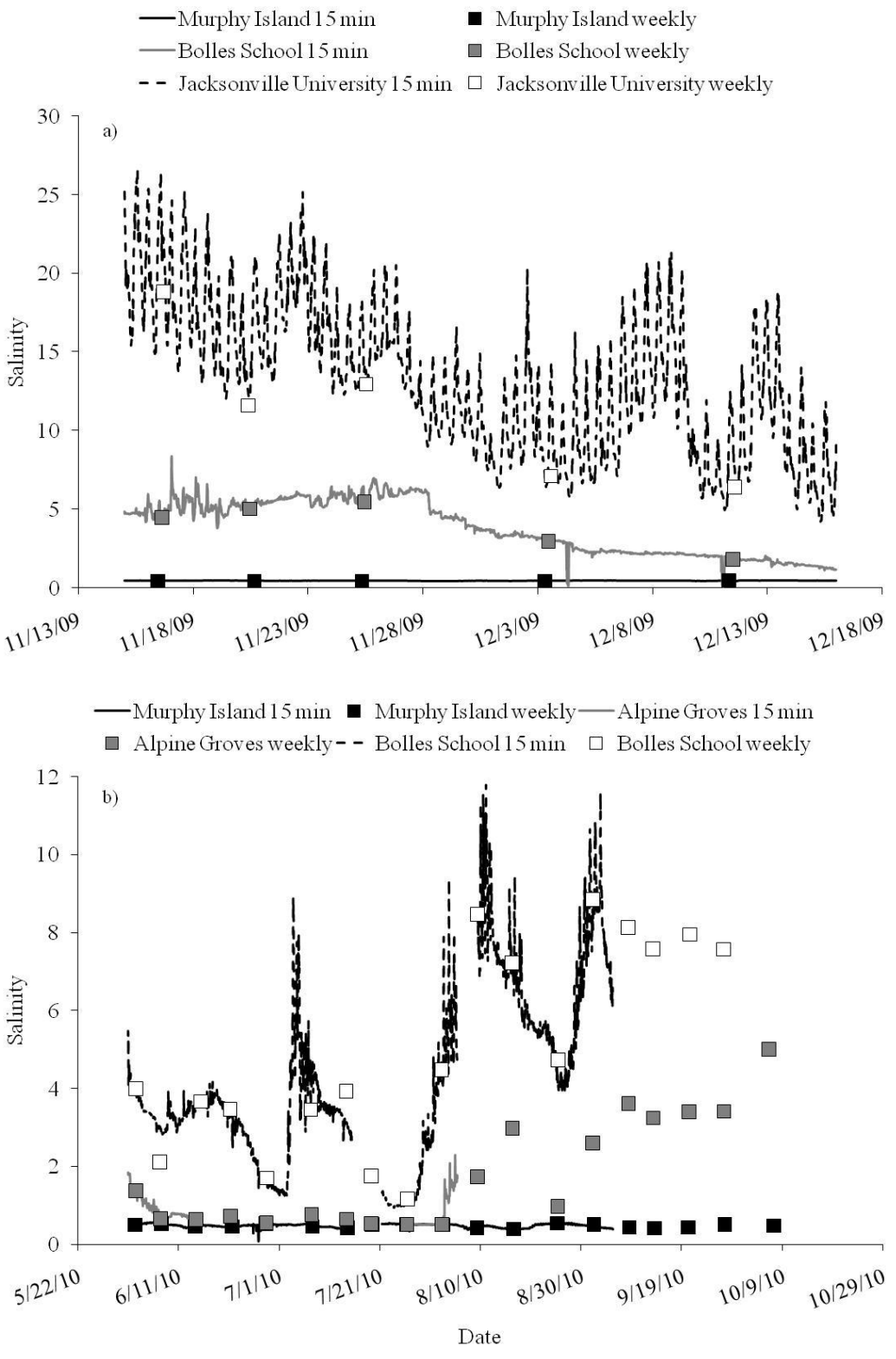

Figure 7. Salinities from weekly and 15-minute sampling for a) the first experiment and b) the second experiment.

Viability of Vallisneria americana

During the first experiment, transplanted *Vallisneria americana* experienced salinities that fell from 18.8 to 6.4 at the Jacksonville University site, decreased from 5.0 to 1.8 at the Bolles School site, and remained around 0.5 at the Murphy Island site. For all cycles, analyses indicated that numbers of ramets and blades varied significantly through time in patterns that differed among the combinations of site of origin and site of treatment (Tables 7 and 8). In some cases, temporal variation among stations within sites also was significant, but the analyses accounted for this variation, and patterns at the site level hold greater biological interest. Due to the relatively balanced design and levels of significance beyond 0.01, these results were considered robust in spite of persistent non-normality and one case of heteroscedasticity (numbers of blades in the 10–12 day cycle; Table 8).

Table 7. Results of tests of assumptions and analyses of variance using $\log_{10}(\text{ramets pot}^{-1} + 1)$ from the first experiment. Ti = time during experiment, with alternating periods of exposure to higher salinity and recovery at lower salinity; Or_Tr = combinations of site of origin and site of treatment, with both sites coinciding for controls; Stn = stations within sites

Cycle	Cochran's	Anderson–Darling	Source	df	SS	MS	*F*	*p*
3–5 days	$p > 0.05$	$p < 0.01$	Time	6	9.2559	1.5426	36.00	< 0.001
			Or_Tr	4	7.6211	1.9053	15.35	0.005
			Stn(Or_Tr)	5	0.6205	0.1241	0.60	0.704
			Pot(Or_Tr * Stn)	70	14.5937	0.2085	10.60	< 0.001
			Time * Or_Tr	24	5.7721	0.2405	5.61	< 0.001
			Time * Stn(Or_Tr)	30	1.2854	0.0429	2.18	< 0.001
			Error	403	7.9250	0.0189		
6–9 days	$p > 0.05$	$p < 0.01$	Time	4	5.0682	1.2671	49.34	< 0.001
			Or_Tr	4	9.8932	2.4733	7.49	0.024
			Stn(Or_Tr)	5	1.6515	0.3303	2.57	0.034
			Pot(Or_Tr * Stn)	70	9.0044	0.1286	9.36	< 0.001
			Time * Or_Tr	16	3.9506	0.2469	9.61	< 0.001
			Time * Stn(Or_Tr)	20	0.5136	0.0257	1.87	0.015
			Error	262	3.6001	0.0137		
10–12 days	$p > 0.05$	$p < 0.01$	Time	2	2.9047	1.4524	71.40	< 0.001
			Or_Tr	4	6.8486	1.7121	28.76	0.001
			Stn(Or_Tr)	5	0.2977	0.0595	0.61	0.696
			Pot(Or_Tr * Stn)	70	6.8880	0.0984	7.03	< 0.001
			Time * Or_Tr	8	2.3871	0.2984	14.67	< 0.001
			Time * Stn(Or_Tr)	10	0.2034	0.0203	1.45	0.165
			Error	119	1.6647	0.0140		
Duration	$p > 0.01$	$p < 0.01$	Time	1	3.3782	3.3782	221.08	< 0.001
			Or_Tr	4	2.1375	0.5344	1.84	0.258
			Stn(Or_Tr)	5	1.4491	0.2898	4.33	0.002
			Pot(Or_Tr * Stn)	70	4.6839	0.0669	3.93	< 0.001
			Time * Or_Tr	4	3.7084	0.9271	60.67	< 0.001
			Time * Stn(Or_Tr)	5	0.0764	0.0153	0.90	0.489
			Error	54	0.9186	0.0170		

Table 8. Results of tests of assumptions and analyses of variance using $\log_{10}(\text{blades pot}^{-1} + 1)$ from the first experiment. Ti = time during experiment, with alternating periods of exposure to higher salinity and recovery at lower salinity; Or_Tr = combinations of site of origin and site of treatment, with both sites coinciding for controls; Stn = stations within sites

Cycle	Cochran's	Anderson–Darling	Source	df	SS	MS	F	p
3–5 days	$p > 0.05$	$p < 0.01$	Ti	6	33.4588	5.5765	56.33	< 0.001
			Or_Tr	4	51.6656	12.9164	29.37	0.001
			Stn(Or_Tr)	5	2.1989	0.4398	0.80	0.553
			Pot(Or_Tr * Stn)	70	38.4848	0.5498	8.83	< 0.001
			Ti * Or_Tr	24	21.2691	0.8862	8.95	< 0.001
			Ti * Stn(Or_Tr)	30	2.9708	0.099	1.59	0.027
			Error	403	25.1033	0.0623		
6–9 days	$p > 0.05$	$p < 0.01$	Ti	4	23.1783	5.7946	158.32	< 0.001
			Or_Tr	4	52.3514	13.0878	17.26	0.004
			Stn(Or_Tr)	5	3.7922	0.7584	2.66	0.029
			Pot(Or_Tr * Stn)	70	19.9304	0.2847	9.25	< 0.001
			Ti * Or_Tr	16	15.852	0.9908	27.07	< 0.001
			Ti * Stn(Or_Tr)	20	0.7326	0.0366	1.19	0.263
			Error	262	8.0606	0.0308		
10–12 days	$p < 0.01$	$p < 0.01$	Ti	2	15.2579	7.6289	684.82	< 0.001
			Or_Tr	4	33.2618	8.3154	149.50	0.000
			Stn(Or_Tr)	5	0.2781	0.0556	0.25	0.940
			Pot(Or_Tr * Stn)	70	15.7251	0.2246	9.63	< 0.001
			Ti * Or_Tr	8	17.9278	2.2410	201.17	< 0.001
			Ti * Stn(Or_Tr)	10	0.1114	0.0111	0.48	0.902
			Error	119	2.7766	0.0233		
Duration	$p > 0.01$	$p < 0.01$	Ti	1	14.5335	14.5335	109.85	< 0.001
			Or_Tr	4	9.1875	2.2969	4.42	0.067
			Stn(Or_Tr)	5	2.5982	0.5197	3.35	0.009
			Pot(Or_Tr * Stn)	70	10.8692	0.1553	3.34	< 0.001
			Ti * Or_Tr	4	15.9022	3.9756	30.05	0.001
			Ti * Stn(Or_Tr)	5	0.6615	0.1323	2.85	0.024
			Error	54	2.5079	0.0464		

Back-transformed means and 95% confidence limits indicated that numbers of ramets and blades decreased primarily in those pots transferred to the Jacksonville University site (Figures 8 and 9). Plants transferred to the Bolles School site from Murphy Island and plants held at Murphy Island as controls for handling stress exhibited little to no loss of ramets and blades. Plants transferred to the Jacksonville University site from Bolles School and controls held at Bolles School did lose ramets and blades, with the losses in controls attributed to the effects of handling on these relatively small and poorly anchored plants. Support for this interpretation comes from comparisons across cycles, with controls at Bolles School exhibiting greater losses for shorter cycles that involved more handling. At the salinities characterizing the Jacksonville University site, there was little evidence of recovery or delayed mortality. For example, the ramets in the 3–5 day cycle had lost nearly all their blades by 25 November 2009, the end of the second period of exposure to higher salinity. Before they were lost, blades turned brown indicating a loss of chlorophyll, and they lost turgor and became flaccid. In addition, significant loss of sediment indicated that rhizomes were lost or less than fully functional.

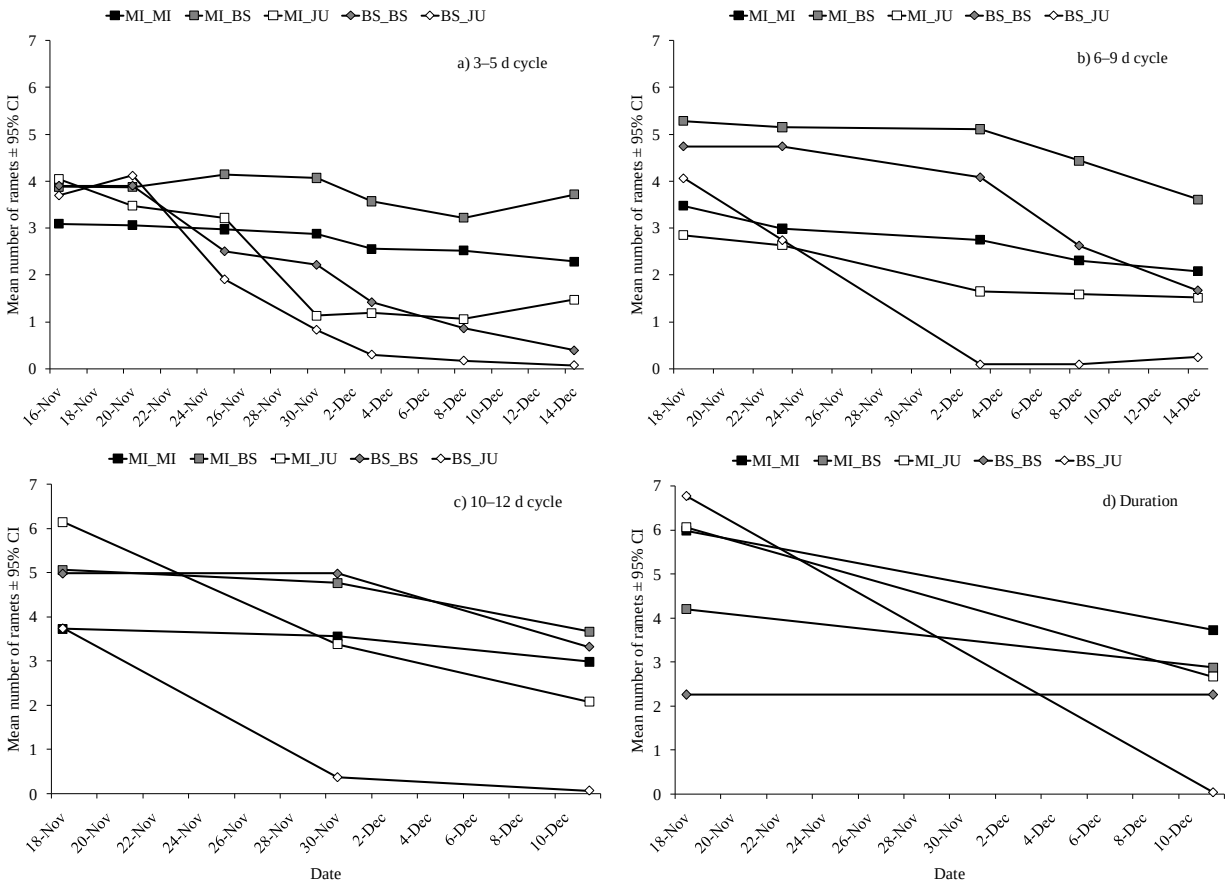

Figure 8. Back-transformed mean number of ramets pot^{-1} ± 95% confidence intervals (CI) across periods of exposure to higher salinity and recovery at lower salinity on a) a 3–5 d cycle, b) a 6–9 d cycle, c) a 10–12 d cycle and d) for the duration of the first experiment. NB: confidence intervals are not visible at this scale; MI_MI, MI_BS, MI_JU, BS_BS and BS_JU = site of origin and site of treatment for pots of *Vallisneria americana*

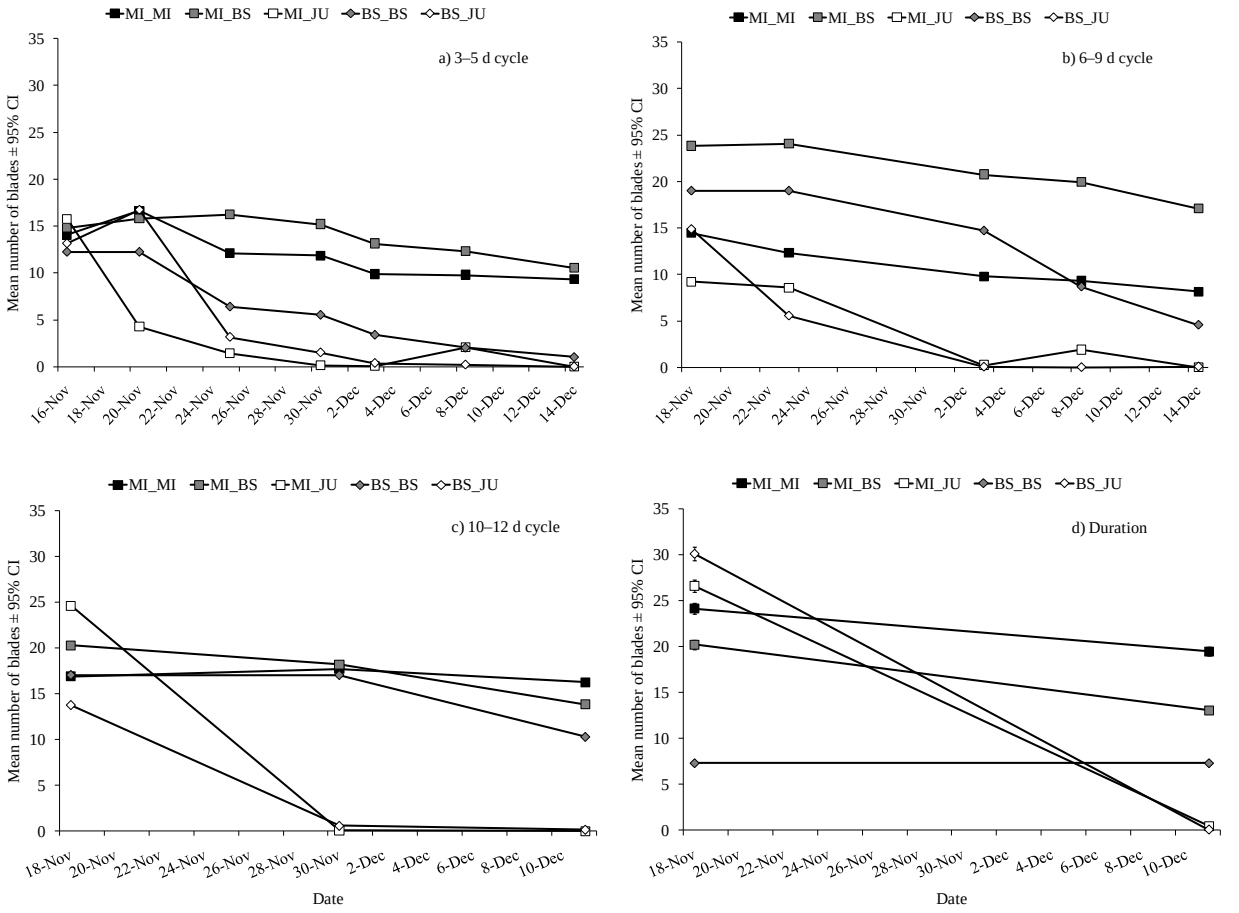

Figure 9. Back-transformed mean number of blades pot^{-1} ± 95% confidence intervals (CI) across periods of exposure to higher salinity and recovery at lower salinity on a) a 3–5 d cycle, b) a 6–9 d cycle, c) a 10–12 d cycle and d) for the duration of the experiment. NB: confidence intervals are not visible at this scale; MI_MI, MI_BS, MI_JU, BS_BS and BS_JU = site of origin and site of treatment for pots of *Vallisneria americana*

During the experiment, plants did not produce reproductive structures, and the coverage of epiphytes remained low and relatively constant (Table 9). Averaged for ramets, over 97% of blades consistently had less than 25% of their surface area covered by epiphytes, and only three ramets exhibited greater than 50% cover of epiphytes across all sites and times. Thus, plants were unlikely to have suffered stress due to shading by epiphytes.

Table 9. Levels of epiphyte cover tallied for sites and times during the first experiment.

Treatment site or Date	Percentage of ramets in epiphyte coverage categories				Total number of ramets
	0–25%	26–50%	51–75%	76–100%	
Murphy Island	98.29	1.71	0.00	0.00	938
Bolles School	98.80	1.07	0.09	0.04	2247
Jacksonville University	98.18	1.82	0.00	0.00	1373
16 November	97.02	2.73	0.00	0.25	403
17 November	96.96	2.61	0.43	0.00	230
18 November	97.55	2.45	0.00	0.00	940
20 November	98.48	1.22	0.30	0.00	328
23 November	100.00	0.00	0.00	0.00	325
25 November	99.63	0.37	0.00	0.00	273
30 November	96.72	3.28	0.00	0.00	519
3 December	99.30	0.70	0.00	0.00	431
8 December	100.00	0.00	0.00	0.00	354
11 December	100.00	0.00	0.00	0.00	439
14 December	100.00	0.00	0.00	0.00	316

In the second experiment, transplanted *Vallisneria americana* experienced salinities that rose from 0.4 to 8.8 at the Bolles School site, increased from 0.4 to 5.0 at the Alpine Groves site, and vacillated between 0.3 and 0.7 at the Murphy Island site. Conservative interpretations of analyses for all cycles indicated that numbers of ramets and blades varied significantly through time in patterns that differed among the stations within the combinations of site of origin and site of treatment (Tables 10 and 11). Due to the relatively balanced design and levels of significance beyond 0.01, these results were considered robust in spite of persistent non-normality and one instance of heteroscedasticity (numbers of blades pot^{-1} in the Duration cycle), with various transformations leading to even greater departures from desired distributions.

Table 10. Results of tests of assumptions and analyses of variance using ramets pot^{-1} from the second experiment. Ti = time during experiment, with alternating periods of exposure to higher salinity and recovery at lower salinity; Or_Tr = combinations of site of origin and site of treatment, with both sites coinciding for controls; Stn = stations within sites

Cycle	Cochran's	Anderson–Darling	Source	df	SS	MS	*F*	*p*
1 week	$p > 0.01$	$p < 0.01$	Ti	18	1699.93	94.44	3.29	< 0.001
			Or_Tr	4	3135.64	783.91	0.41	0.794
			Stn(Or_Tr)	5	9501.70	1900.34	5.58	< 0.001
			Pot(Or_Tr * Stn)	70	23834.78	340.50	49.91	< 0.001
			Or_Tr	72	1909.61	26.52	0.92	0.636
			Ti * Stn(Or_Tr)	90	2585.74	28.73	4.21	< 0.001
			Error	1258	8582.09	6.82		
2 weeks	$p > 0.05$	$p < 0.01$	Ti	8	683.90	85.49	3.79	0.002
			Or_Tr	4	1636.74	409.19	0.59	0.686
			Stn(Or_Tr)	5	3471.47	694.29	4.54	0.001
			Pot(Or_Tr * Stn)	70	10713.18	153.05	26.91	< 0.001
			Or_Tr	32	1299.81	40.62	1.80	0.039
			Ti * Stn(Or_Tr)	40	902.60	22.56	3.97	< 0.001
			Error	559	3179.69	5.69		
3 weeks	$p > 0.05$	$p < 0.01$	Ti	6	645.87	107.64	6.92	< 0.001
			Or_Tr	4	285.76	71.44	0.20	0.928
			Stn(Or_Tr)	5	1788.89	357.78	2.82	0.022
			Pot(Or_Tr * Stn)	70	8882.43	126.89	18.67	< 0.001
			Or_Tr	24	774.54	32.27	2.07	0.030
			Ti * Stn(Or_Tr)	30	466.73	15.56	2.29	< 0.001
			Error	418	2840.57	6.80		
Duration	$p > 0.05$	$p < 0.01$	Ti	3	300.06	100.02	1.92	0.169
			Or_Tr	4	2274.14	568.53	1.49	0.332
			Stn(Or_Tr)	5	1907.52	381.50	6.17	< 0.001
			Pot(Or_Tr * Stn)	70	4326.72	61.81	6.64	< 0.001
			Or_Tr	12	619.49	51.62	0.99	0.497
			Ti * Stn(Or_Tr)	15	779.55	51.97	5.58	< 0.001
			Error	210	1954.66	9.31		

Table 11. Results of tests of assumptions and analyses of variance using blades pot^{-1} from the second experiment. Ti = time during experiment, with alternating periods of exposure to higher salinity and recovery at lower salinity; Or_Tr = combinations of site of origin and site of treatment, with both sites coinciding for controls; Stn = stations within sites

Cycle	Cochran's	Anderson–Darling	Source	df	SS	MS	*F*	*p*
1 week	$p > 0.05$	$p < 0.01$	Ti	18	93572.0	5198.0	4.18	< 0.001
			Or_Tr	4	82006.0	20501.0	0.37	0.822
			Stn(Or_Tr)	5	277387.0	55477.0	4.31	0.002
			Pot(Or_Tr * Stn)	70	901414.0	12877.0	49.18	< 0.001
			Or_Tr	72	94428.0	1311.0	1.05	0.406
			Ti * Stn(Or_Tr)	90	112070.0	1245.0	4.76	< 0.001
			Error	1258	329354.0	261.8		
2 weeks	$p > 0.01$	$p < 0.01$	Ti	8	63692.2	7961.5	8.21	< 0.001
			Or_Tr	4	69164.5	17291.1	0.65	0.649
			Stn(Or_Tr)	5	132030.3	26406.1	4.31	0.002
			Pot(Or_Tr * Stn)	70	429190.7	6131.3	24.93	< 0.001
			Or_Tr	32	48031.2	1501.0	1.55	0.095
			Ti * Stn(Or_Tr)	40	38781.3	969.5	3.94	< 0.001
			Error	559	137713.8	245.9		
3 weeks	$p > 0.01$	$p < 0.01$	Ti	6	65747.0	10957.8	6.92	< 0.001
			Or_Tr	4	14276.2	3569.0	0.20	0.928
			Stn(Or_Tr)	5	58591.0	11718.2	2.82	0.022
			Pot(Or_Tr * Stn)	70	382386.8	5462.7	18.67	< 0.001
			Or_Tr	24	37320.7	1555.0	2.07	0.030
			Ti * Stn(Or_Tr)	30	20368.8	679.0	2.29	< 0.001
			Error	418	112742.6	269.7		
Duration	$p < 0.01$	$p < 0.01$	Ti	3	16497.8	5499.3	1.34	0.298
			Or_Tr	4	85885.7	21471.4	1.88	0.253
			Stn(Or_Tr)	5	57232.2	11446.4	4.24	0.002
			Pot(Or_Tr * Stn)	70	188849.5	2697.9	6.67	< 0.001
			Or_Tr	12	39601.6	3300.1	0.81	0.642
			Ti * Stn(Or_Tr)	15	61405.3	4093.7	10.12	< 0.001
			Error	210	84924.1	404.4		

In comparison to the values from the first experiment, means and standard errors indicated that numbers of ramets and blades in pots remained relatively constant throughout the second experiment (Figures 9–12). As indicated by the significant interactions in the ANOVAs, mean numbers of ramets and blades differed among replicate pots allocated to different combinations of site and station within site. In most cases, these differences persisted throughout the experiment, with the most obvious exception being controls on the 1-week cycle at Alpine Groves, which lost ramets and blades to become more similar to controls at Murphy Island. This result suggested slight handling stress at this intensity of manipulation. Regardless, there was no evidence of a decrease in viability similar to that observed in plants transferred to Jacksonville University (salinities of 6.4 to 18.8).

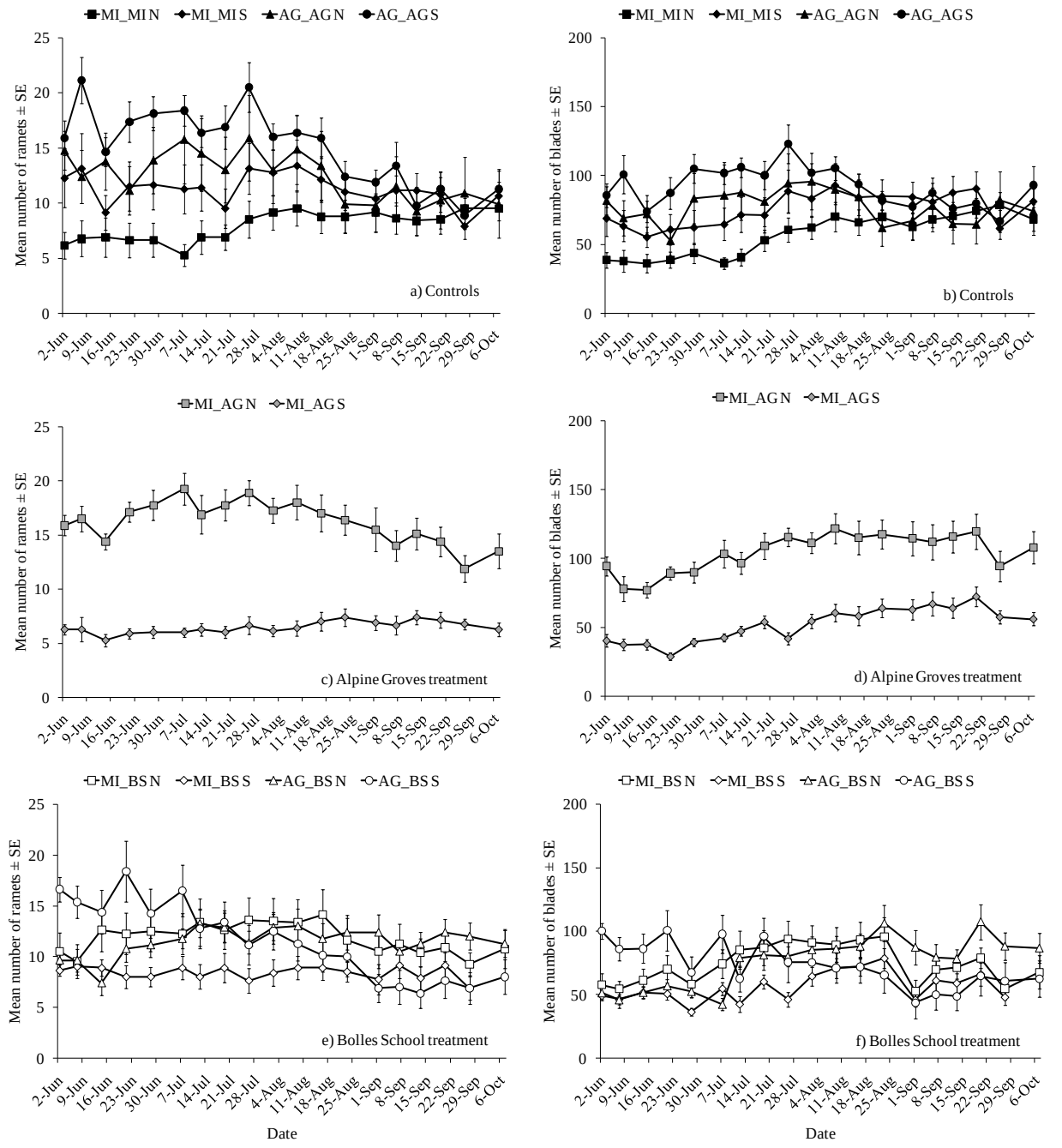

Figure 10. Mean number of ramets and blades pot^{-1} ± standard errors (SE) for a) and b) control plants held at their sites of origin and plants subjected to alternating 1-week periods of exposure to higher salinity and recovery at lower salinity for c) and d) plants transferred from Murphy Island to Alpine Groves and e) and f) plants transferred from Murphy Island and Alpine Groves to Bolles School. MI_MI, MI_AG, MI_BS, AG_AG and AG_BS = site of origin and site of treatment for pots of *Vallisneria americana*; N and S = north and south stations within sites

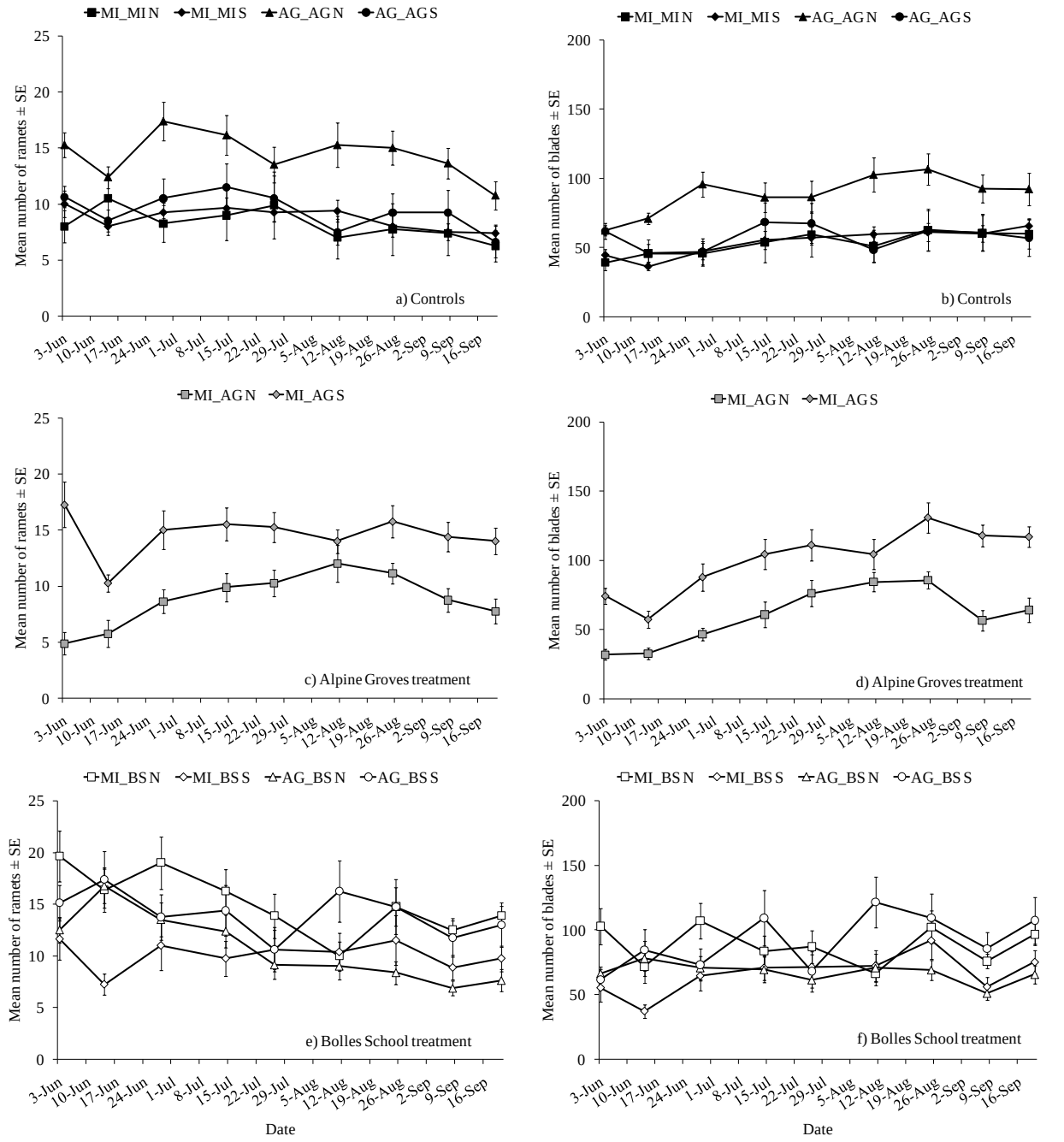

Figure 11. Mean number of ramets and blades pot^{-1} ± standard errors (SE) for a) and b) control plants held at their sites of origin and plants subjected to alternating 2-week periods of exposure to higher salinity and recovery at lower salinity for c) and d) plants transferred from Murphy Island to Alpine Groves and e) and f) plants transferred from Murphy Island and Alpine Groves to Bolles School. MI_MI, MI_AG, MI_BS, AG_AG and AG_BS = site of origin and site of treatment for pots of *Vallisneria americana*; N and S = north and south stations within sites

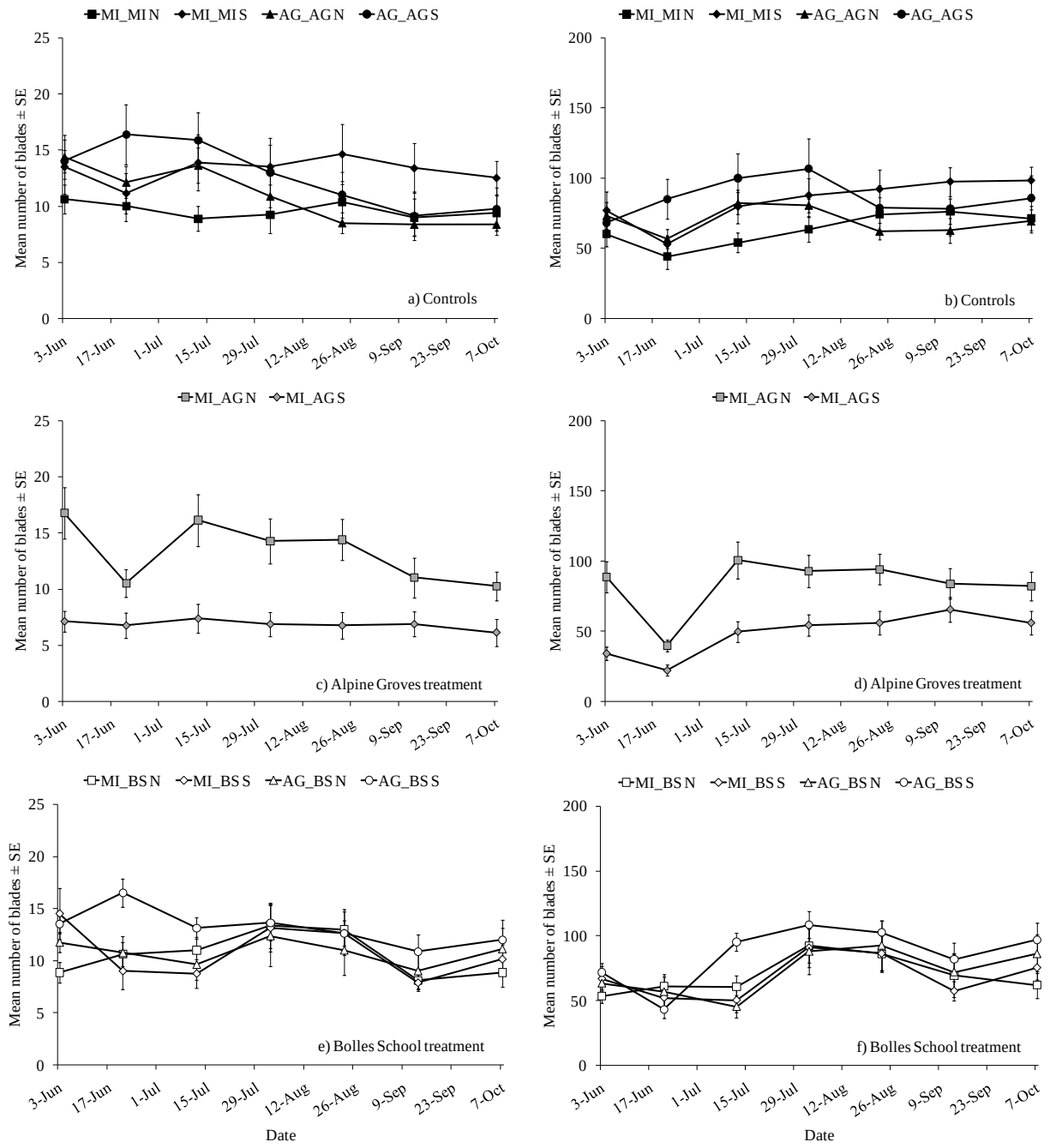

Figure 12. Mean number of ramets and blades pot^{-1} ± standard errors (SE) for a) and b) control plants held at their sites of origin and plants subjected to alternating 3-week periods of exposure to higher salinity and recovery at lower salinity for c) and d) plants transferred from Murphy Island to Alpine Groves and e) and f) plants transferred from Murphy Island and Alpine Groves to Bolles School. MI_MI, MI_AG, MI_BS, AG_AG and AG_BS = site of origin and site of treatment for pots of *Vallisneria americana*; N and S = north and south stations within sites

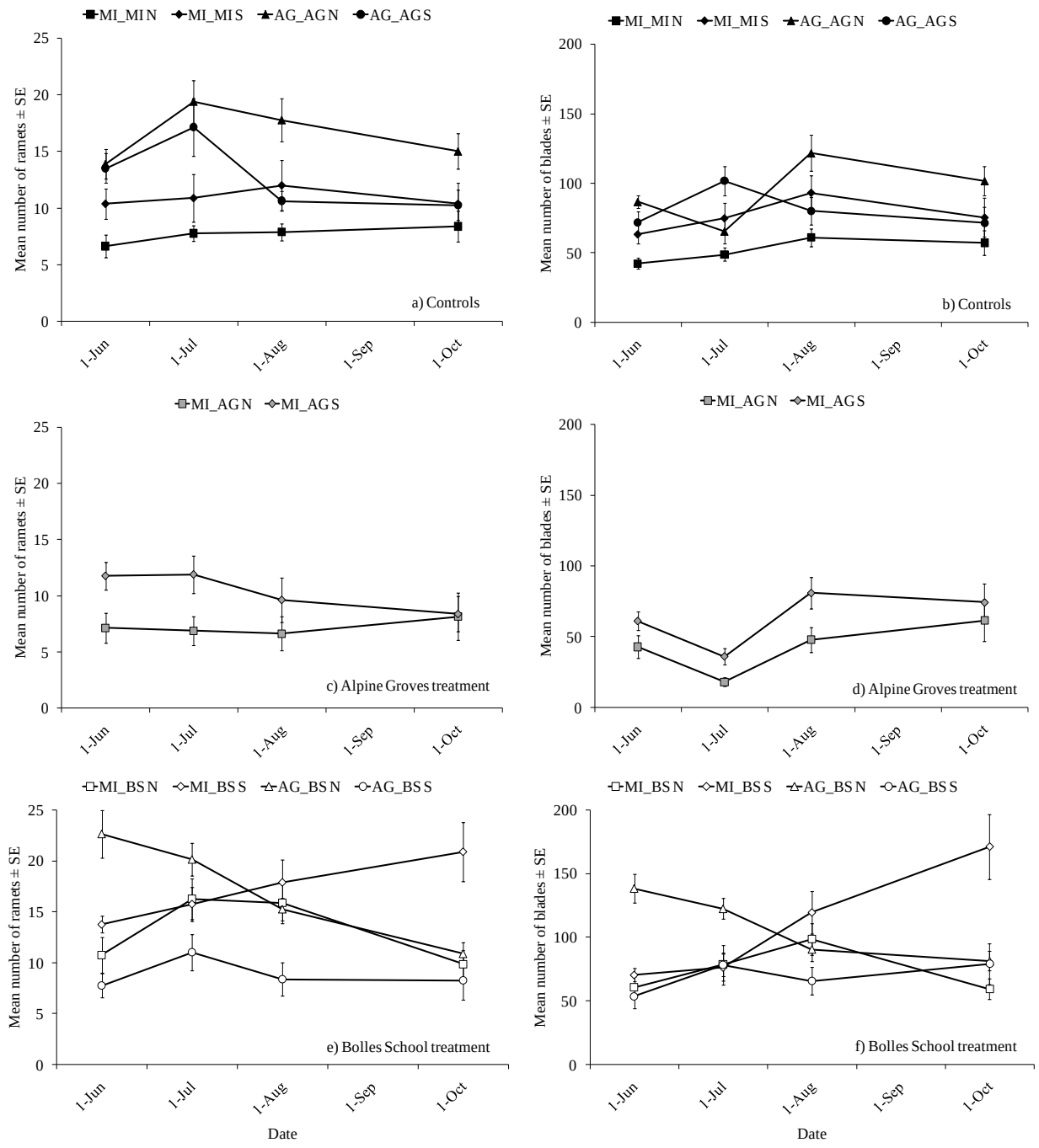

Figure 13. Mean number of ramets and blades pot^{-1} ± standard errors (SE) for a) and b) control plants held at their sites of origin and plants subjected to higher salinity for the 18-week duration of the experiment at c) and d) Alpine Groves and e) and f) Bolles School. MI_MI, MI_AG, MI_BS, AG_AG and AG_BS = site of origin and site of treatment for pots of *Vallisneria americana*; N and S = north and south stations within sites

In comparison to the first experiment, plants in the second experiment exhibited greater epiphyte coverage at the start of the experiment, initially had reproductive structures, and began to produce new reproductive structures at the end of the experiment (Tables 12 and 13). The percentage of ramets having epiphyte coverage of 26–50% was highest at Murphy Island and lowest at Alpine Groves. Concentrations of chlorophyll *a* in the water column also were highest at Murphy Island, which suggested suitable conditions for the growth of various algae. Overall, plants were subjected to shading from epiphytes primarily at Murphy Island and Bolles School early in the experiment. At the end of the experiment, reproductive structures appeared on plants in pots and nearby undisturbed plants at the Murphy Island site, which indicated that plants remained healthy throughout the 18-week experiment. In fact, some blades, particularly those subjected to the least stress at Murphy Island, elongated noticeably during the experiment (Figure 13).

Table 12. Levels of epiphyte cover tallied for sites and times during the second experiment.

Treatment site or Date	Percentage of ramets in epiphyte coverage categories				Total number of ramets
	0–25%	26–50%	51–75%	76–100%	
Murphy Island	73.92	23.45	2.50	0.14	2124
Alpine Groves	94.65	5.12	0.23	0.00	4394
Bolles School	83.90	15.01	1.02	0.07	4423
31 May–6 June	77.33	22.33	0.34	0.00	1178
7–13 June	78.65	19.57	1.78	0.00	281
14–20 June	80.39	18.55	1.06	0.00	566
21–27 June	80.55	17.41	2.05	0.00	586
28 June–4 July	83.72	15.93	0.35	0.00	565
5–11 July	93.41	6.59	0.00	0.00	577
12–18 July	86.55	12.40	0.94	0.12	855
19–25 July	98.95	1.05	0.00	0.00	286
26 July–1 August	77.78	20.97	1.08	0.18	558
2–8 August	78.01	19.55	2.27	0.17	573
9–15 August	73.64	21.27	4.55	0.55	550
16–22 August	78.96	18.17	2.88	0.00	556
23–29 August	80.34	18.46	1.21	0.00	829
30 August–5 September	92.45	7.55	0.00	0.00	265
6–12 September	99.63	0.37	0.00	0.00	546
13–19 September	99.45	0.55	0.00	0.00	544
20–26 September	100.00	0.00	0.00	0.00	542
27 September–3 October	100.00	0.00	0.00	0.00	269
4–10 October	99.88	0.00	0.12	0.00	815

Table 13. Presence of reproductive structures tallied for sites and times during the second experiment.

Treatment site or Date	Number of		
	female flowers	male flowers	seed pods
Murphy Island	3	0	0
Alpine Groves	12	4	3
Bolles School	18	0	1
31 May–6 June	19	1	4
7–13 June	5	0	0
14–20 June	5	1	0
21–27 June	4	0	0
28 June–4 July	0	0	0
5–11 July	0	0	0
12–18 July	0	0	0
19–25 July	0	0	0
26 July–1 August	0	0	0
2–8 August	0	0	0
9–15 August	0	0	0
16–22 August	0	0	0
23–29 August	0	0	0
30 August–5 September	0	0	0
6–12 September	0	0	0
13–19 September	0	0	0
20–26 September	0	0	0
27 September–3 October	0	0	0
4–10 October	0	2	0

Figure 14. Photograph illustrating blade elongation observed for some plants.

The second experiment included assays of stress enzymes as an additional measure of changes related to periods of exposure to salinity and recovery. Results of analyses of variance indicated that both total antioxidant capacity and catalase activity varied significantly among times but not among combinations of sites of origin and treatment or their interaction with time for all cycles except the 3-week cycle where the interaction terms were significant for both measures (Table 14).

Table 14. Results of tests of assumptions and analyses of variance using $\log_{10}$(enzyme activity) measured during the second experiment. Antioxidant = total antioxidant capacity; Catalase = catalase activity; Ti = time during experiment, with alternating periods of exposure to higher salinity and recovery at lower salinity; Or_Tr = combinations of site of origin and site of treatment, with both sites coinciding for controls

Cycle	Parameter	Cochran's	Anderson–Darling	Source	df	SS	MS	*F*	*p*
1 week	Antioxidant	$p < 0.01$	$p < 0.01$	Ti	18	2.29175	0.12732	5.78	0.000
				Or_Tr	4	0.21507	0.05377	2.44	0.052
				Ti * Or_Tr	72	1.31153	0.01822	0.83	0.801
				Error	95	2.09353	0.02204		
	Catalase	$p > 0.05$	$p > 0.05$	Ti	18	2.29090	0.12727	8.94	0.000
				Or_Tr	4	0.19930	0.04983	3.50	0.010
				Ti * Or_Tr	72	1.15172	0.01600	1.12	0.295
				Error	95	1.35237	0.01424		
2 weeks	Antioxidant	$p > 0.05$	$p > 0.05$	Ti	8	0.51609	0.06451	3.26	0.005
				Or_Tr	4	0.16619	0.04155	2.10	0.097
				Ti * Or_Tr	32	0.52179	0.01631	0.82	0.714
				Error	45	0.89029	0.01978		
	Catalase	$p > 0.05$	$p > 0.05$	Ti	8	0.35450	0.04431	2.28	0.038
				Or_Tr	4	0.16544	0.04136	2.13	0.092
				Ti * Or_Tr	32	0.51789	0.01618	0.83	0.701
				Error	45	0.87284	0.01940		
3 weeks	Antioxidant	$p > 0.05$	$p < 0.01$	Ti	6	1.83670	0.30612	13.33	0.000
				Or_Tr	4	0.20422	0.05105	2.22	0.086
				Ti * Or_Tr	24	2.98374	0.12432	5.41	0.000
				Error	35	0.80360	0.02296		
	Catalase	$p > 0.05$	$p < 0.01$	Ti	6	1.61123	0.26854	20.07	0.000
				Or_Tr	4	0.38405	0.09601	7.18	0.000
				Ti * Or_Tr	24	1.75506	0.07313	5.47	0.000
				Error	35	0.46822	0.01338		
Duration	Antioxidant	$p > 0.05$	$p > 0.01$	Ti	3	1.19785	0.39928	11.08	0.000
				Or_Tr	4	0.07074	0.01768	0.49	0.742
				Ti * Or_Tr	12	0.49144	0.04095	1.14	0.378
				Error	24	0.86467	0.03603		
	Catalase	$p > 0.05$	$p > 0.05$	Ti	3	0.61417	0.20472	12.79	0.000
				Or_Tr	4	0.04157	0.01039	0.65	0.633
				Ti * Or_Tr	12	0.34289	0.02857	1.78	0.110
				Error	24	0.38430	0.01601		

Back-transformed means and 95% confidence intervals indicated that total antioxidant capacity and catalase activity were relatively high for sampling periods before 26 July 2010 (Figures 14–17). In general, measures of enzyme activity did not track increases and decreases in salinity closely. Furthermore, water temperatures did not differ significantly during the experiment; therefore, heat stress was unlikely. Although blades for analysis of enzyme activity generally were collected as soon as plants were brought aboard the boat, high levels of activity could have been caused by handling stress during the early part of the experiment, especially given air temperatures that were above 32°C. Relatively constant levels of enzyme activity after the initial weeks of the experiment indicated that *Vallisneria americana* plants were healthy regardless of exposure to higher salinities at differing cycles.

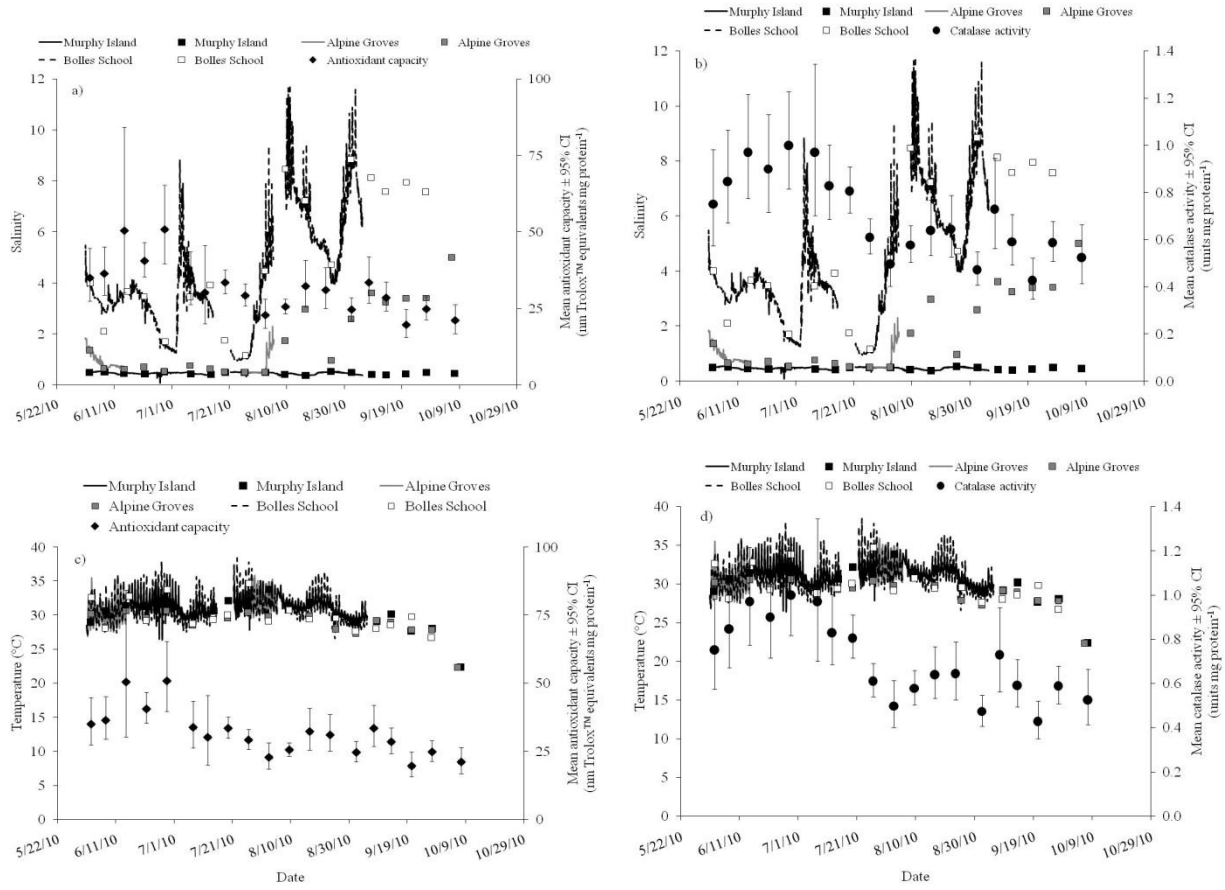

Figure 15. Back-transformed mean total antioxidant capacities and catalase activities ± 95% confidence intervals for the 1-week cycle versus a) and b) salinity and c) and d) temperature. red, yellow and green lines = data collected each 15 min; red, yellow and green boxes = data collected weekly

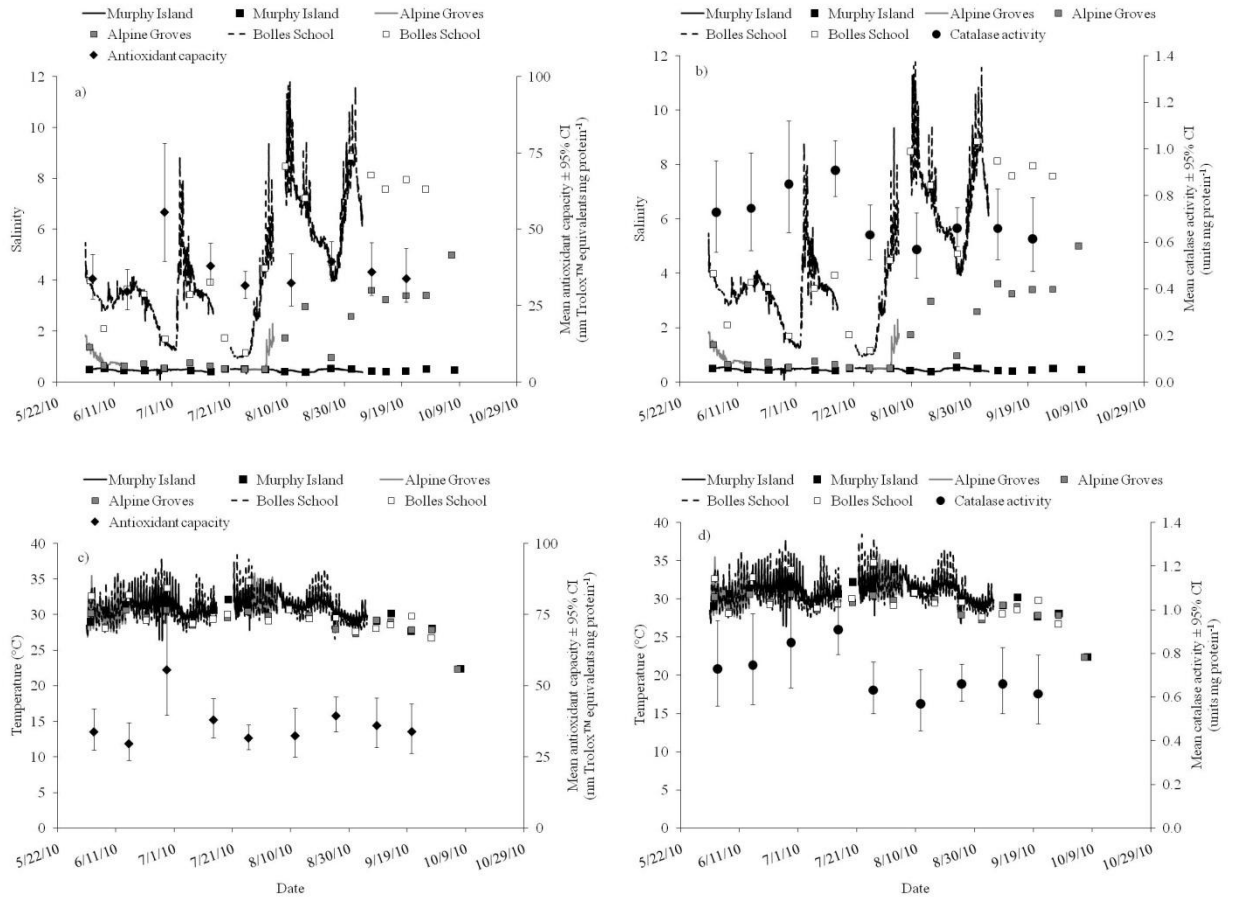

Figure 16. Back-transformed mean total antioxidant capacities and catalase activities ± 95% confidence intervals for the 2-week cycle versus a) and b) salinity and c) and d) temperature. red, yellow and green lines = data collected each 15 min; red, yellow and green boxes = data collected weekly

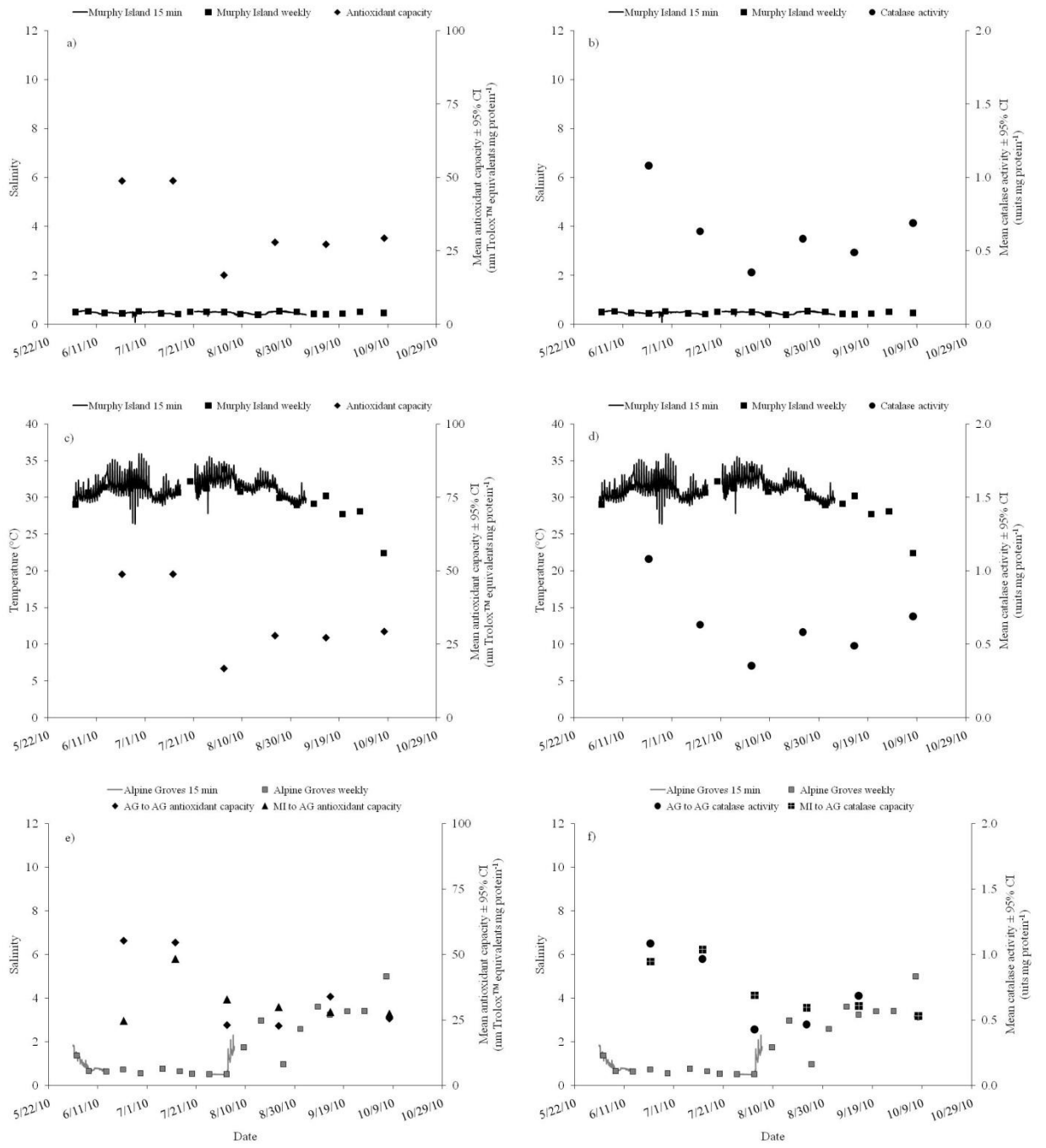

Figure 17. Back-transformed mean total antioxidant capacities and catalase activities ± 95% confidence intervals for the 3-week cycle versus a), b), e), f), i) and j) salinity and c), d), g), k), j) and l) temperature. NB confidence intervals are not visible at this scale; red, yellow and green lines = data collected each 15 min; red, yellow and green boxes = data collected weekly

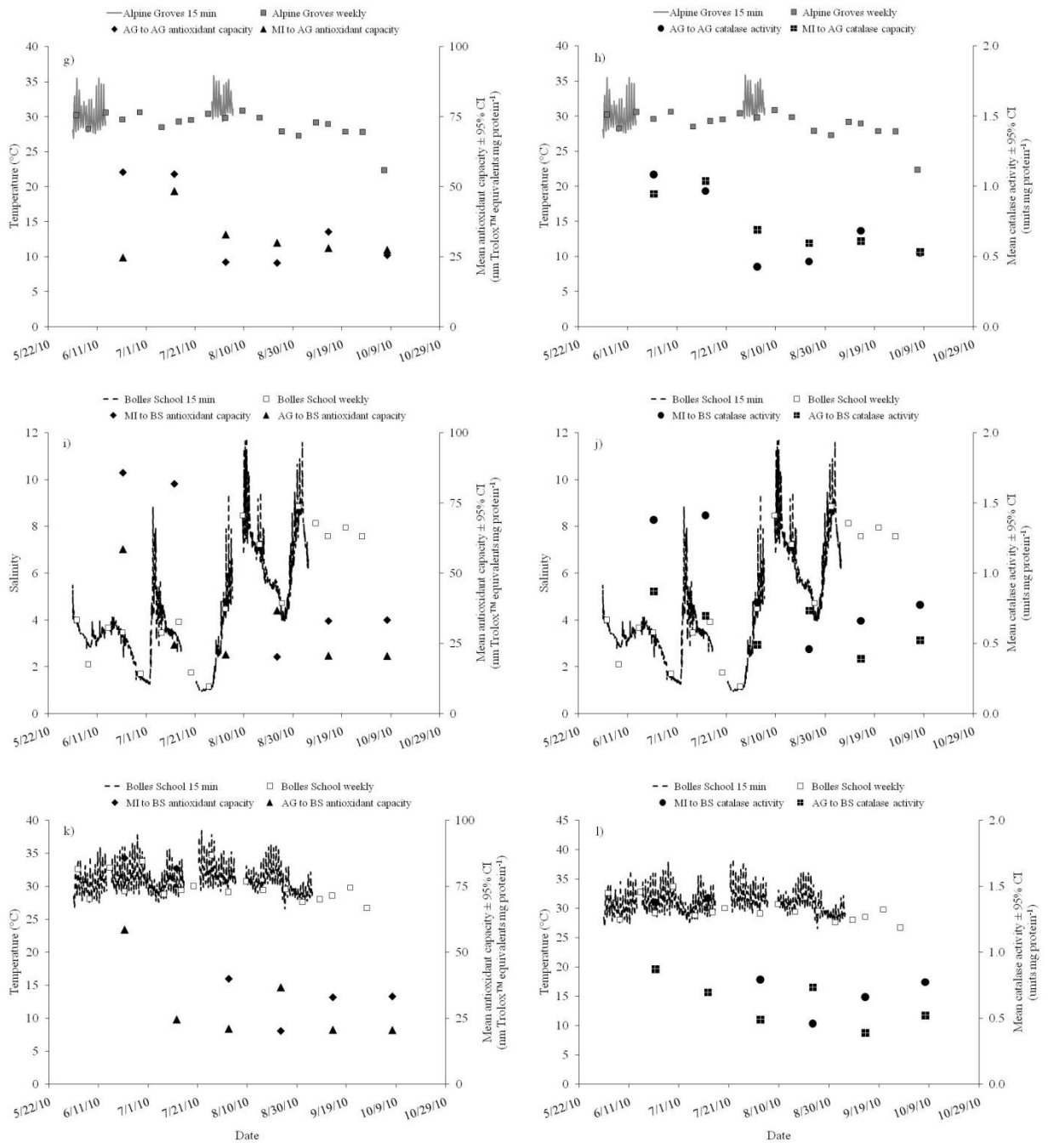

Figure 17 (cont.). Back-transformed mean total antioxidant capacities and catalase activities ± 95% confidence intervals for the 3-week cycle versus a), b), e), f), i) and j) salinity and c), d), g), k), j) and l) temperature. NB confidence intervals are not visible at this scale; red, yellow and green lines = data collected each 15 min; red, yellow and green boxes = data collected weekly

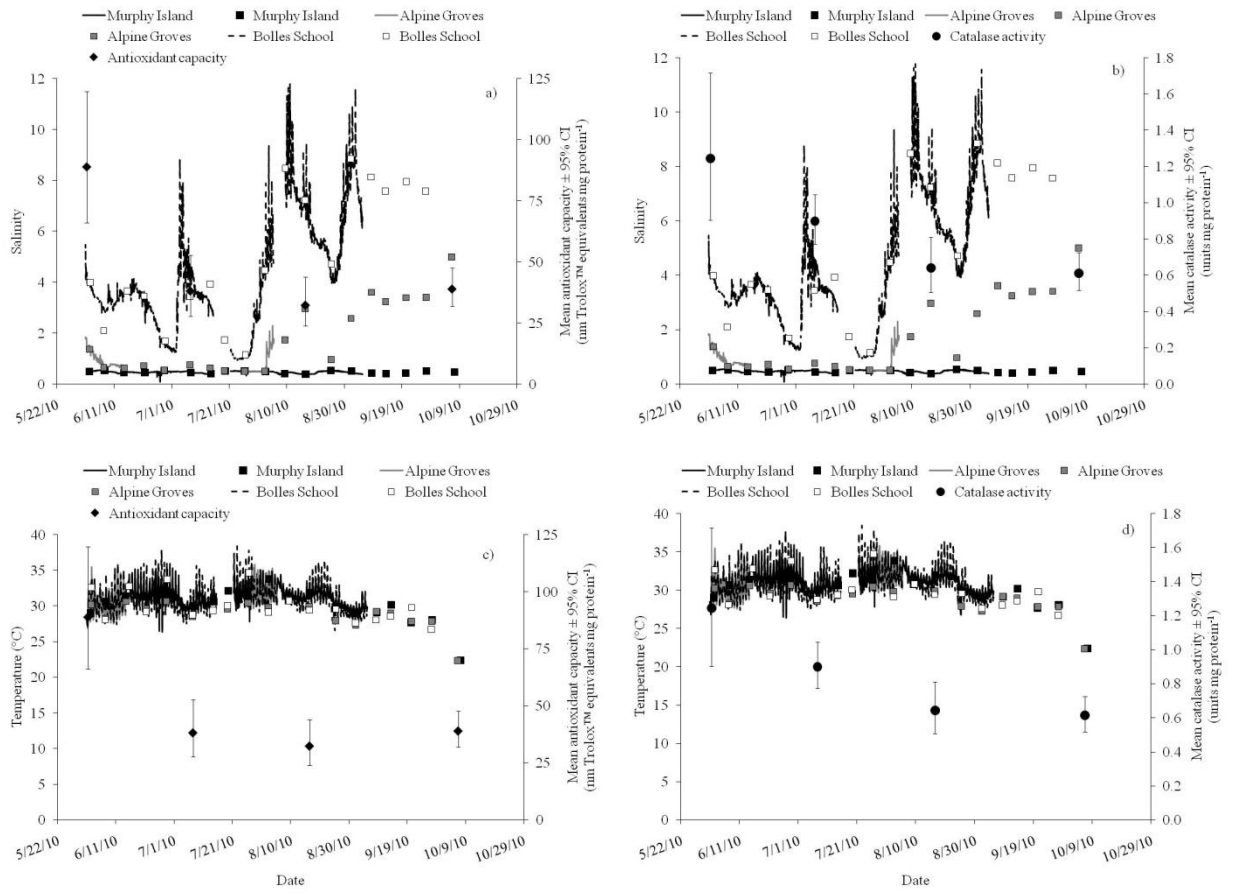

Figure 18. Back-transformed mean total antioxidant capacities and catalase activities ± 95% confidence intervals for plants held at sites for the 18-week duration of the experiment versus a) and b) salinity and c) and d) temperature. red, yellow and green lines = data collected each 15 min; red, yellow and green boxes = data collected weekly

Discussion

The most obvious effects on the viability of *Vallisneria americana* correlated with differences in salinity. Two potentially key influences, i.e., light climates and temperatures, were similar and within the physiological tolerances of *V. americana* at all experimental sites. After an exposure period of only 3–5 d, exposure to a salinity of 18 resulted in significant loss of blades and ramets, with no signs of viable belowground structures or recovery. In contrast, salinity of up to 8.8 produced no consistent decrease in viability in either experiment across periods of exposure varying from 3 to 128 d. At salinities of 8.8 or less, changes in numbers of ramets or blades, and changes in enzymes did not indicate clear cycles of stress and recovery that paralleled experimental manipulations.

The experiments demonstrated that *in situ* transplants can provide useful data on the physiological tolerances. Future efforts should ensure that similarly robust ramets are chosen to reduce variation in persistence due to differing rhizome mats. Stress enzymes may yet provide a valuable metric of the effects of salinity before blades and ramets are lost, but any experiment measuring enzymes should include a treatment guaranteed to produce salinity stress, which can be difficult in a field experiment. Further experimentation to document maximum durations of exposure to various levels of salinity

tolerated by *Vallisneria americana* would add significantly to an understanding of this plant. Experiments wherein the recovery of damaged ramets is tracked over longer periods also would add value.

Based on these results, the existing conceptual model for salinity stress on *Vallisneria americana* can be confirmed and modified (Figure 18). Increased mortality at the Jacksonville University site confirmed that *V. americana* did not tolerate salinity of 18 for even 3 d. In contrast, plants held at Bolles School indicated that salinities of 8.8 were tolerated for up to 18 cycles of exposure and recovery over a period of up to 128 d, which resulted in an extension of the no effect prediction at salinities below 10. Overall, results suggested that managing water withdrawals to avoid intrusions of salinity above 10 should protect *V. americana* and the natural resources that it supports.

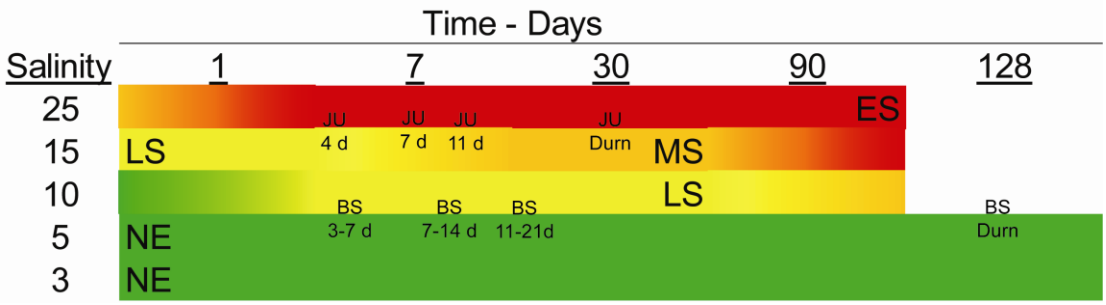

Figure 19. Impacts of salinity on *Vallisneria americana* (original figure from Dobberfuhl et al. 2009). ES = extreme stress; MS = moderate stress; LS = low stress; NE = no effect; JU = Jacksonville University; BS = Bolles School

References

Antweiler, R. C., and H. E. Taylor. 2008. Evaluation of statistical treatments of left-censored environmental data using coincident uncensored data sets: I. summary statistics. Environ. Sci. Technol. **42**: 3732–3738.

Boustany, R. G., T. C. Michot and R. F. Moss. 2010. Effects of salinity and light on biomass and growth of *Vallisneria americana* from the lower St. Johns River, FL, USA. Wetl. Ecol. Manag. **18**: 203–217.

Dobberfuhl, D. R., R. Mattson, K. Moore, L. Morris and R. Virnstein. 2009. An examination of potential littoral zone effects as a result of surface water withdrawals. pp.: 553–610. In: Lowe, E. F. and L. E. Battoe (eds.). Alternative water supply cumulative impact assessment interim report. Draft report from the St. Johns River Water Management District. 781 pp.

Doering, P. H., R. H. Chamberlain and J. M. McMunigal. 2001. Effects of simulated saltwater intrusions on the growth and survival of wild celery, *Vallisneria americana*, from the Caloosahatchee Estuary (south Florida). Estuaries **24**: 894–903.

Frazer, T. K., S. K. Notestein, C. A. Jacoby, C. J. Littles, S. R. Keller and R. A. Swett. 2006. Effects of storm-induced salinity changes on submersed aquatic vegetation in Kings Bay, Florida. Estuaries Coasts **29**: 943–953.

French, G. T., and K. A. Moore. 2003. Interactive effects of light and salinity stress on growth, reproduction, and photosynthetic capabilities of *Vallisneria americana* (wild celery). Estuaries **26**: 1255–1268.

Jarvis, J. C., and K. A. Moore. 2008. Influence of environmental factors on *Vallisneria americana* seed germination. Aquat. Bot. **88**: 283–294.

Kraemer, G. P., R. H. Chamberlain, P. H. Doering, A. D. Steinman and M. D. Hanisak. Physiological responses of transplants of the freshwater angiosperm *Vallisneria americana* along a salinity gradient in the Caloosahatchee Estuary (southwestern Florida). Estuaries **22**: 138–148.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. **26**: 1231–1237.

Sagan, J. J. 2009. Summary of submerged aquatic vegetation (SAV) status within the lower St. Johns River: 1996–2007. Final report to the St. Johns River Water Management District contract number SJ45514. 167 pp.