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Effects of Simulated Saltwater Intrusions on the Growth and Survival of Wild Celery, *Vallisneria americana*, from the Caloosahatchee Estuary (South Florida)

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ABSTRACT: The effects of simulated saltwater intrusions on the growth and survival of the freshwater angiosperm, *Vallisneria americana* Michx., from the Caloosahatchee estuary (southwest Florida, USA) were examined experimentally using indoor mesocosms. Intrusions were simulated by raising salinity in the mesocosms to 18‰ for varying durations and then returning the salinity to 3‰. In separate experiments, exposures of short duration (1, 5, 11, and 20 d) and long duration (20, 30, 50, and 70 d) were examined. Plants held at a constant 3‰ served as controls. Mortality was proportional to the duration of exposure. Statistically significant ($p < 0.05$) losses of blades and shoots occurred at exposures of 20 d or longer, although during a 1-mo recovery period at 3‰ viable plants survived the 70-d exposure to 18‰. Expressed as a percentage of initial levels, the extent of recovery after 1 mo was proportional to duration of exposure. *V. americana* can survive the salinity stress associated with most intrusions of salt water in the upper Caloosahatchee estuary.

Introduction

Beds of submerged aquatic angiosperms are ecologically important in estuarine and marine systems because they provide habitat for many benthic and pelagic organisms, stabilize sediments, improve water quality, and can form the basis of a detrital food web (Kemp et al. 1984; Thayer et al. 1984; Fonseca and Fisher 1986; Carter et al. 1988; Killgore et al. 1989; Zieman and Zieman 1989; Lubbers et al. 1990). No species of submerged aquatic angiosperm is strictly limited to waters of estuarine salinities, and marine species dominate higher salinity regions (> 20‰) while salt-tolerant freshwater species inhabit the lower salinity regions (Kemp et al. 1984). Establishing the effects of salinity on the growth, survival, and reproduction of these species is key to understanding their distribution and abundance in estuaries.

Wild celery, *Vallisneria americana* Michx., is a salt-tolerant freshwater species that occurs in the fresh, oligohaline, and mesohaline reaches of estuaries in the eastern United States (Bourn 1932; Lowden 1982). *V. americana* is dioecious, perennial, and capable of extensive clonal growth through the formation of stolons (Lovett-Doust and Laporte 1991). Northern populations overwinter as a dormant winter bud buried in the sediments (Titus and Hoover 1991). In south Florida, populations do not completely die back in winter and actively growing plants may be found all year (Dawes and Lawrence 1989).

In sub-tropical South Florida, there is a prominent seasonal variation in salinity driven by wet season (May–October) and dry season (November–April) differences in rainfall and runoff. During the winter dry season, significant up-estuary saltwater intrusions of varying strength and duration can occur. The tolerance of *V. americana* to salinities of different strength has been investigated previously (Doering et al. 1999). Here we examine the tolerance of *V. americana* to simulated saltwater intrusions of constant strength but varying duration and the re-growth or recovery of *V. americana* following these intrusions.

Study Area

The Caloosahatchee estuary is located on the southwest coast of Florida, USA (Fig. 1). The major source of freshwater is the Caloosahatchee River, which runs 65 km from Lake Okeechobee to the head of the estuary at the Franklin Lock and Dam (S-79, Fig. 1). Beds of *V. americana* can occur up to 30 km downstream of S-79 but grow most luxuriantly upstream of the bridges at Ft. Myers, especially in the upper estuary around Beautiful Island (Fig. 1; Chamberlain and Doering 1998). When conditions are favorable, *V. americana* exhibits a

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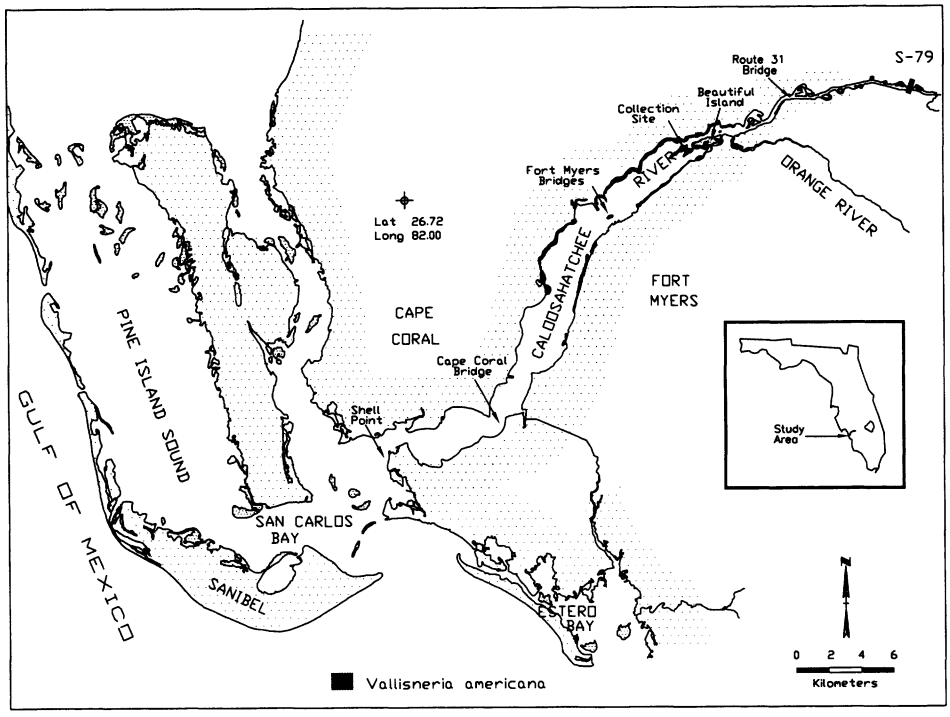

Fig. 1. Distribution of *Vallisneria americana* in the Caloosahatchee estuary. Also shown is the collection site and the location of salinity recorders at the Route 31 and Ft. Myers Bridges.

seasonal pattern of growth, with highest biomass achieved in the late summer, flowering in the late summer-early fall, and a winter decline in biomass (Bortone and Turpin 2000). Like other populations in Florida, *V. americana* does not completely die back in winter (Dawes and Lawrence 1989). Although viable rosettes can normally be found in the Caloosahatchee any time of the year, the density of *V. americana* begins to decline as salinity rises above 10‰ (Chamberlain and Doering 1998).

In the upper estuary, temporal fluctuations in salinity are largely driven by freshwater discharge at S-79 (Fig. 2). During periods of high discharge, usually during the summer wet season, the system turns fresh. During periods of low discharge, usually during the winter dry season, salt water intrudes up the estuary. Daily rates of change can be on the order of 2‰ d^{-1} at the Ft. Myers bridges (Doering et al. 1999). Analysis of long-term records of salinity (Fig. 2) at the Ft. Myers bridges downstream of Beautiful Island and at the Rt. 31 Bridge, upstream of the island, reveal the character of saltwater intrusions into the upper estuary. Saltwater intrusions ($\geq$ 10‰) may last for over 100 d, but almost all (> 90%) are less than 70 d and median durations are relatively short (5–12 d, Table 1). Peak salinities average 13–14‰ which is near *V. americana*'s upper tolerance limit for growth (15‰; Doering et al. 1999) but may exceed this (Table 1). Most peak salinities are less than 18‰ (75% at the Ft. Myers bridges and 100% at the Route 31 Bridge). The peak salinity achieved during an intrusion is related to the duration of the intrusion at both the Rt. 31 Bridge (Spearman's r = 0.864, n = 11, $p < 0.05$) and at Ft. Myers (Spearman's r = 0.837, n = 25, $p < 0.05$). This relationship probably reflects the cumulative effect of successive tides mixing seawater further up the estuary.

Materials and Methods

Saltwater intrusions were simulated using an experimental mesocosm facility located indoors at an aquarium facility at the Gumbo Limbo Nature Center in Boca Raton, Florida. Plants were grown in ten cylindrical mesocosm tanks (1.3 m in diameter × 1 m deep) filled with water to a depth of 60 cm (volume = 800 l). A 1,000 Watt metal halide lamp, kept on a L:D photoperiod of 12:12 h, supplied light to each tank. Salinity was controlled by mixing appropriate volumes of freshwater and salt water (total volume = 114 l) from each of two head tanks (one for salt water, one for freshwater) located above each mesocosm. Volume in individual head tanks was controlled using standpipe drains and salinity was manipulated by changing relative standpipe height in the two head tanks. Head tanks were alternately filled and emptied

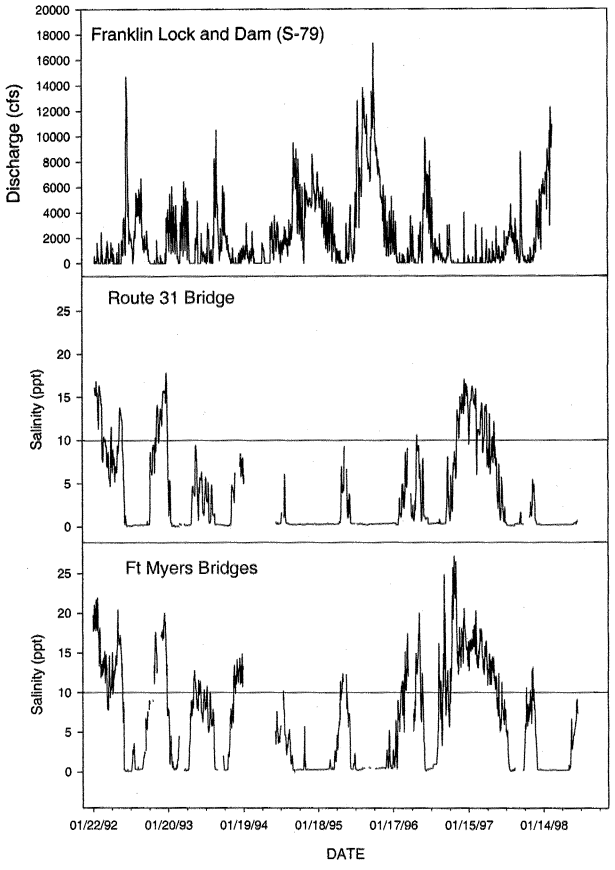

Fig. 2. Daily average freshwater discharge at S-79 and salinity at the Route 31 and Ft. Myers Bridges from records maintained by the South Florida Water Management District.

into the mesocosms using solenoid valves controlled by timers. Mixing was achieved by delivering water from the head tanks to a mesocosm through a common pipe and by a 7-watt submersible pump located in the mesocosm itself. Water was delivered in a series of 114-l pulses, replacing the volume in the mesocosms 3 times per day. Salt water was pumped from the Atlantic Ocean. Tap water, passed through a series of charcoal towers and filters (20 micron pore size) to remove chlorine, was used as a source of freshwater.

Two experiments were conducted to examine the response of *V. americana* to, and its recovery from, exposure to varying periods of high (18‰) salinity. The first experiment lasted from March 24, 1997 to May 28, 1997 (66 d) and examined short-term exposures of 1, 5, 11, and 20 d duration. The second experiment lasted from January 5, 1998 to May 1, 1998 (117 d) and examined longer term exposures of 20, 30, 50, and 70 d. To examine the ability *V. americana* to recover, treatments in both experiments were observed for a minimum of 30 d following exposure.

TABLE 1. Characteristics of salt water intrusions at two stations in the upper Caloosahatchee estuary: Route 31 Bridge (Rt. 31) and Ft. Myers Bridges (FTM). Saltwater intrusions defined as beginning when salinity rises above 10‰ and ending when salinity falls below 10‰.

		Peak Salinity (‰)			Duration (d)		
Station	n	Mean	Median	Max	Mean	Median	Range
Rt. 31	11	13.4	13.10	17.8	25.3	5	3–126
FTM	25	14.6	12.7	27.1	26.8	12	1–225

Collection of Plants

V. americana were collected from a site adjacent to Beautiful Island in the Caloosahatchee estuary (Fig. 1). Plastic, rectangular tubs (14 cm H × 24 cm L × 15 cm W) were filled with sieved (0.25 cm^2 mesh) sediment in the field. Plants, including roots, were carefully removed by hand from the sediment, placed in ice chests, covered with water from the site, and transported back to the laboratory on the day of collection. Plants were held overnight, in the dark, at 3‰ in a mesocosm tank. On the following day, rosettes were planted in the tubs by burying roots in the sediment. Tubs were distributed among the mesocosms (5 tubs per mesocosm) and held at 3‰ for a 4–7 week acclimation period. Plants for the short-term experiment were collected on February 25, 1997 and for the long-term experiment on November 10, 1997. Salinity was 3‰ at the collection site on both occasions.

Planting and acclimation of rosettes for the short-term experiment followed procedures previously established by Doering et al. (1999). In the short-term experiment, 4 rosettes were planted in each tub and new shoots produced during acclimation were removed to ensure consistent initial conditions. To improve estimates of mortality in the long-term experiment, 6 rosettes per tub were planted and new shoots produced during acclimation were not removed.

Salinity Experiments

In both experiments, two mesocosms were assigned to each exposure treatment. Exposure consisted of raising the salinity from 3‰ to 18‰ at a rate of 2–3‰ d^{-1}, holding it at 18‰ for the appropriate duration and then lowering the salinity back to 3‰ at a rate of 2–3‰ d^{-1} (Table 2). Following return to 3‰, treatments were observed for a recovery period of approximately 1 mo (28–30 d). An experiment was terminated at the end of the recovery period for the longest exposure treatment (Table 2). Both experiments included a control treatment in which salinity remained at 3‰.

The following non-destructive measurements were taken over time in both experiments: number

TABLE 2. Time course of each experiment. Values are day of experiment. In both experiments, measurements were taken in each treatment and controls on Day 1 when lowering salinity back to 3‰ was complete, at the end of the recovery period, and at the end of the experiment. Additional measurements also were taken over time in the long-term experiment.

Treatment	Raise Salinity	Exposure to 18‰	Lower Salinity	End of Recovery	End of Experiment
Short-term experiment					
Control	—	—	—	—	66
1 day	1–9	10	11–19	47	66
5 day	1–9	10–14	15–23	51	66
11 day	1–9	10–20	21–29	57	66
20 day	1–9	10–29	30–38	66	66
Long-term experiment					
Control	—	—	—	—	117
20 day	1–9	10–29	30–38	67	117
30 day	1–9	10–39	40–48	78	117
50 day	1–9	10–59	60–68	96	117
70 day	1–9	10–79	80–88	117	117

of shoots per tub, number of blades per tub, number of blades per shoot, and blade length of 20 randomly selected blades from each tub. Sampling was more frequent during the long-term experiment. Treatments in both experiments were sampled at the beginning of exposure (salinity starts to rise from 3‰), the end of exposure (salinity returns to 3‰), after 1 mo of recovery (28–30 d after the end of exposure), and at the end of an experiment.

Once a week, tanks were scrubbed to remove wall growth and any epiphytic growth was gently removed by hand from plant blades. Although not quantified, epiphytic growth was not extensive and no differences between treatments were apparent. This routine cleaning procedure ensured that epiphytic growth did not confound the experimental results. Salinity and temperature were monitored hourly in each mesocosm by a YSI 600XL data sonde connected to a data logger. Photosynthetically active radiation (PAR) was checked weekly with a LICOR spherical quantum sensor and data logger and adjusted to 425–550 μmol photons m^{-2} s^{-1} at the bottom of the tank by raising or lowering the lamp. This light intensity is well above the I_{sat} of 200 μmol photons m^{-2} s^{-1} for *V. americana* (Harley and Findlay 1994).

Statistical Analysis

The treatments, including control, were compared at three times using a two factor repeated measures analysis of variance (Winer 1971). The factors were exposure treatments with five levels and time with three levels. Levels of the time factor were the beginning of exposure (Day 1), the end of exposure (when salinity returned to 3‰), and after 1 mo of recovery. Because the mesocosms were the experimental units in this analysis, data were averaged across tubs within each mesocosm. This procedure yielded one observation per mesocosm per sample date and two observations per treatment per sample date. Statistically significant ($p < 0.05$) differences between main effect means (treatment or time) were further evaluated using Fisher's LSD test (Winer 1971). Statistically significant interactions between treatment and time were evaluated following Winer (1971) and Keppel (1973). Differences between times at each level of treatment were examined using Fisher's LSD test (Winer 1971), while differences between treatments at each time were examined using a two-way ANOVA with mesocosms nested within the treatment factor (Keppel 1973). Significant differences between treatment means detected by this ANOVA were evaluated with the LSD test. Because the control was not exposed to high salinity, samplings for the end of exposure and 1 mo of recovery were arbitrarily selected to match those for the shortest salinity exposure treatment (1 or 20 d, depending on the experiment). This procedure artificially reduced differences between controls and treatments and therefore provides conservative estimates of these differences.

In each experiment, all treatments except those of longest duration (20 or 70 d) were followed for more than 1 mo after exposure. A second repeated measures ANOVA (treatment × sample date) was conducted to determine the relationship between treatments at the end of the experiment (Day 66 and 117 for the short-term and long-term experiments) and if further recovery to initial levels had occurred in any of the treatments. Dates included those in each experiment upon which all treatments were sampled. Significant main effects and interactions were evaluated as previously described.

Rate of Recovery

To compare rates of recovery, post-exposure shoot counts were modeled using the exponential growth equation ($N_t = N_o e^{rt}$, where N_o = number of shoots at time 0, N_t = the same at time t, and r

= the exponential growth coefficient) after shoot counts were log e transformed to linearize growth curves. Growth coefficient slopes (r) from the different treatments were compared using analysis of covariance (Winer 1971). Two hypotheses were tested using appropriate ANCOVA models: whether slopes in the treatments were different from zero and whether slopes in the treatments were equal to each other. After visual inspection of graphs, post-exposure periods of time over which exponential growth appeared to occur were chosen for analysis. Post-exposure growth in the short-term experiment was sufficient for comparison of all treatments. In the 70-d treatment of the long-term experiment, there were too few data to calculate growth rates. All statistical analyses were conducted using SAS Version 6 (SAS 1989).

Results

With few exceptions, measured salinities over the course of both experiments were maintained within ± 1‰ of nominal treatment salinities (Fig. 3). Failure of the saltwater pumps on day 50 of the long-term experiment caused salinity to drop to about 11‰ for a brief period in the 50-d and 70-d treatments. Malfunction of a solenoid valve on day 93 caused salinity to rise to about 10‰ in one mesocosm (30-d treatment). Water temperatures were higher during the short-term experiment (range of mesocosm means 24.4–26.1°C) than during the long-term experiment (range of mesocosm means 20.3–24.8°C).

Exposure and Recovery after 1 Month

The repeated measures analysis of variance comparing treatments at the beginning of exposure, at the end of exposure, and after 1 mo of recovery revealed no statistical differences between treatments at the beginning of exposure in either experiment (Tables 3 and 4). In both experiments, ANOVA results for blades were nearly the same as for shoots. Number of blades in each mesocosm varied with the number of shoots in both experiments ($\bar{x}$ ± SD Pearson's r = 0.915 ± 0.084, n = 20 mesocosms, $p < 0.05$ in all cases) and this relationship accounts for the similarity of ANOVA results.

In the short-term experiment, only exposure to 18‰ for 20 d resulted in a significant loss of blades and shoots, relative to initial levels (Table 3). After the 1-mo recovery period, shoots and blades had returned to initial levels. No mortality, resulting from exposure, was observed in the other treatments; initial levels and those measured after exposure were similar. By the end of recovery, number of blades and shoots either remained unchanged (11-d treatment) or surpassed initial lev-

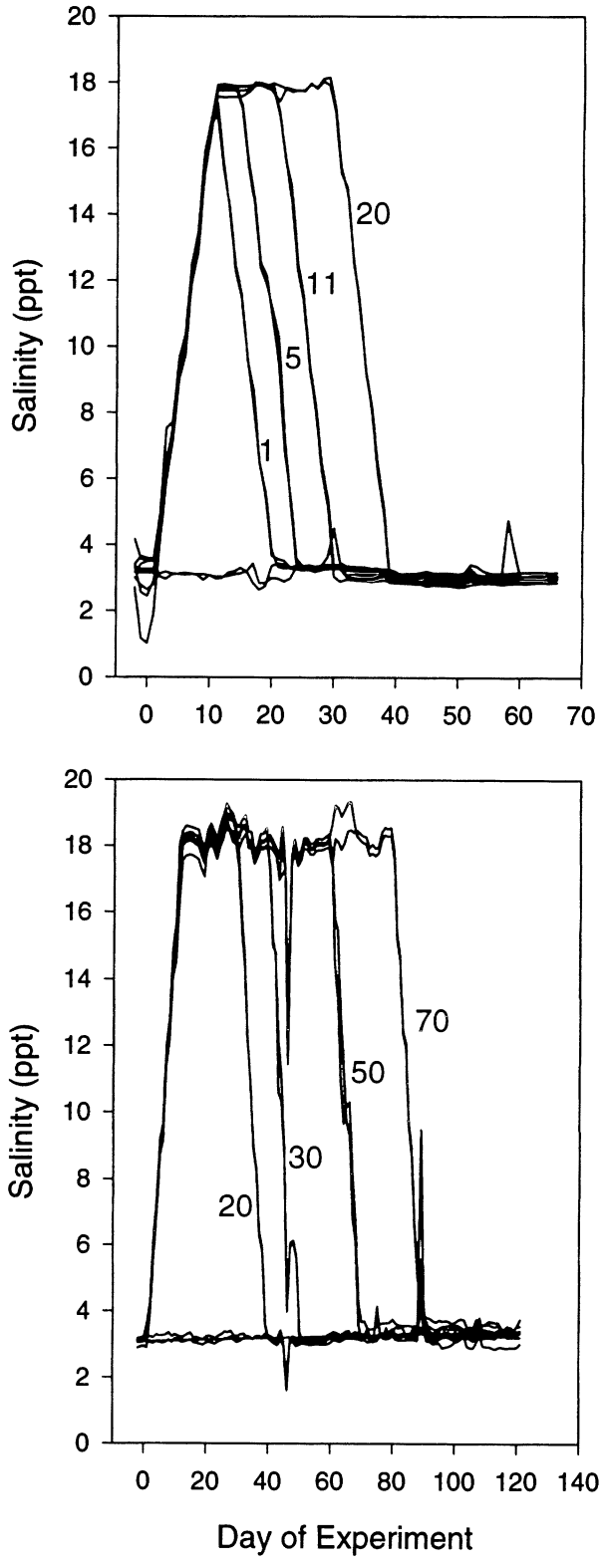

Fig. 3. Time course of salinity in the mesocosms during the two experiments. Numbers refer to duration of exposure to a salinity of 18‰.

TABLE 3. Short-term exposure experiment. Results of repeated measures ANOVA for mean blade length (cm) and mean number of blades, shoots, and blades per shoot. Letters indicate results of Fisher's LSD test. Means with the same letters are not statistically different at $p < 0.05$. Capitol letters compare times within a treatment (down a column). Small letters compare the five treatments at a particular time (across a row). Absence of letters indicates that there were no statistical differences associated with duration of exposure to 18‰. Means calculated by first averaging across tubs in a mesocosm and then averaging the values (n = 2) for the mesocosms.

	Control	1 day	5 day	11 day	20 day
Blades					
Initial	$30.9^{B,a}$	$30.1^{B,a}$	$33.1^{B,a}$	$33.2^{A,a}$	$32.2^{A,a}$
Exposure	$45.7^{B,a}$	$32.2^{B,b}$	$30.2^{B,b}$	$26.8^{A,b}$	$17.3^{B,c}$
Month	$87.6^{A,a}$	$63.9^{A,b}$	$47.2^{A,bc}$	$30.6^{A,cd}$	$25.2^{AB,d}$
Shoots					
Initial	$4.0^{B,a}$	$4.0^{B,a}$	$4.0^{B,a}$	$4.0^{A,a}$	$4.0^{A,a}$
Exposure	$5.3^{B,a}$	$4.6^{B,ab}$	$3.6^{B,bc}$	$3.4^{A,c}$	$2.1^{B,d}$
Month	$12.0^{A,a}$	$8.6^{A,b}$	$6.7^{A,bc}$	$4.8^{A,c}$	$3.6^{AB,c}$
Blades per shoot					
Initial	7.7	7.5	8.3	8.3	8.0
Exposure	8.8	7.2	8.4	8.0	6.9
Month	7.4	7.6	7.6	6.1	6.7
Blade length					
Initial	3.6	3.2	3.3	3.5	3.4
Exposure	2.9	2.9	2.8	2.3	1.9
Month	3.9	4.2	3.8	3.2	2.6

TABLE 4. Long-term exposure experiment. Results of repeated measures ANOVA for mean blade length and mean number of blades, shoots and blades/shoot. Letters indicate results of Fisher's LSD test. Means with the same letters are not statistically different at $p < 0.05$. Capitol letters compare times within a treatment (down a column). Small letters compare the five treatments at a particular time (across a row). Absence of letters indicates that there were no statistical differences associated with duration of exposure to 18‰. Means calculated by first averaging across tubs in a mesocosm and then averaging the values (n = 2) for the mesocosms.

	Control	20 day	30 day	50 day	70 day
Blades					
Initial	$78.7^{B,a}$	$89.3^{A,a}$	$91.7^{A,a}$	$93.9^{A,a}$	$77.3^{A,a}$
Exposure	$102.5^{AB,a}$	$67.9^{A,ab}$	$40.1^{B,bc}$	$22.0^{B,c}$	$8.4^{B,c}$
Month	$129.7^{A,a}$	$90.3^{A,ab}$	$41.3^{B,bc}$	$20.4^{B,c}$	$10.4^{B,c}$
Shoots					
Initial	$16.1^{A,a}$	$17.7^{A,a}$	$16.6^{A,a}$	$19.10^{A,a}$	$15.7^{A,a}$
Exposure	$15.4^{A,a}$	$13.3^{B,a}$	$9.7^{B,ab}$	$5.7^{B,bc}$	$3.4^{B,c}$
Month	$18.3^{A,a}$	$13.5^{B,ab}$	$6.2^{B,bc}$	$3.3^{B,c}$	$2.0^{B,c}$
Blades per shoot					
Initial	$4.9^{B,a}$	$5.1^{B,a}$	$5.5^{B,a}$	$5.0^{B,a}$	$5.0^{B,a}$
Exposure	$6.8^{A,a}$	$4.1^{C,b}$	$4.0^{C,b}$	$3.2^{C,bc}$	$2.3^{C,c}$
Month	$7.1^{A,a}$	$5.7^{A,a}$	$6.9^{A,a}$	$6.3^{A,a}$	$5.8^{A,a}$
Blade length					
Initial	3.1	3.1	2.8	2.9	3.3
Exposure	2.1	1.9	1.6	1.0	0.88
Month	3.4	2.7	2.5	2.2	1.9

els (Control, 1-d, 5-d treatments, Table 3). During the short-term experiment, no statistical effects of duration of exposure on number of blades per shoot were detected (Table 3). Blade length varied similarly over time in all treatments (including the control), declining upon exposure and increasing by the end of the recovery period.

In the 30, 50, and 70-d treatments of the long-term experiment, exposure to 18‰ caused a significant loss of blades and shoots which was not recouped after 1 mo of recovery. In the 20-d treatment, shoots showed a similar pattern, but number of blades did not change significantly (Table 4). Mean number of shoots did not change in the control, but number of blades increased significantly between the initial measurement and the end of recovery.

While the number of blades per shoot increased in the control, exposure to 18‰ caused a significant decrease in all other treatments (Table 4). After recovery, blades per shoot had surpassed initial levels in all exposure treatments. This growth of new blades establishes the viability of plants following exposure, especially in the 30, 50, and 70-d treatments where numbers of shoots and blades did not recover to initial levels. Results for blade length were similar to those in the short-term experiment.

At the end of exposure in both experiments, numbers of shoots and blades declined as duration of exposure increased. When expressed as a percentage of initial numbers remaining after exposure, data from both experiments fell on the same regression lines (Fig. 4).

After 1 mo of recovery at 3‰, the pattern of decreasing numbers of blades and shoots with increasing duration of exposure persisted in both experiments (Tables 3 and 4). Even short-term exposures (1, 5, and 11 d), which caused no mortality, retarded the accumulation of blades and shoots relative to controls (Table 3). The percent of initial blades and shoots present after 1 mo of recovery was also proportional to the duration of exposure to 18‰ (Fig. 4).

Further Recovery

Analysis of the short-term experiment by repeated measures ANOVA (treatment × sample date) revealed that by the end (Day 66) of the short-term experiment, only the 1-d treatment had numbers of blades and shoots similar to the control (Fig. 5). Although the remaining exposure treatments had recovered to initial levels after 1 mo, at the end of the experiment they still had fewer blades and shoots than the control ($p < 0.05$). The number of blades per shoot did not vary between treatments on any day of the experiment (repeated measures ANOVA, $p > 0.05$).

Throughout most of the short-term experiment, longest blades were found in the control or 1-d treatment (Fig. 5). Because of high variability, statistical differences were rarely detected on a given

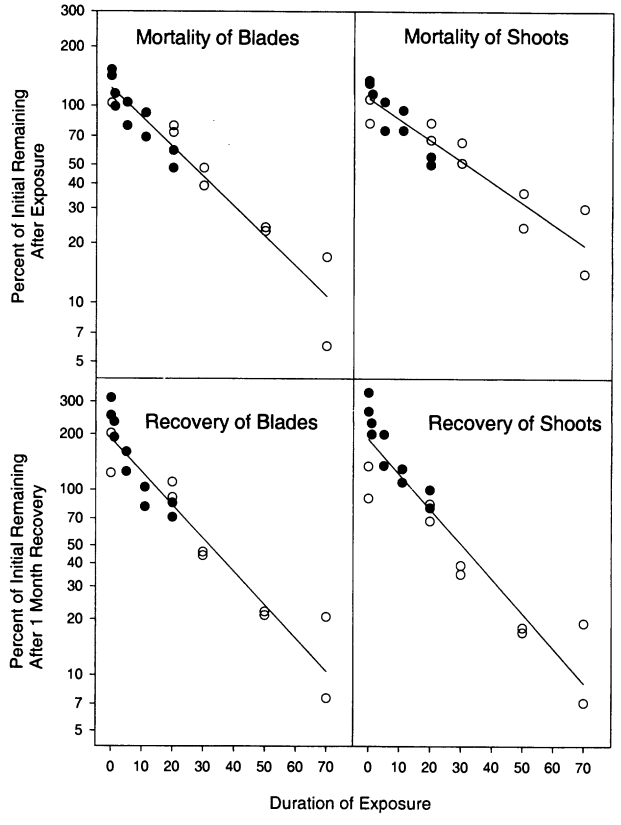

Fig. 4. Mortality and recovery of blades and shoots as a function of duration of exposure to 18‰. Closed circles = short-term experiment, open circles = long-term experiment. Each point represents one mesocosm.

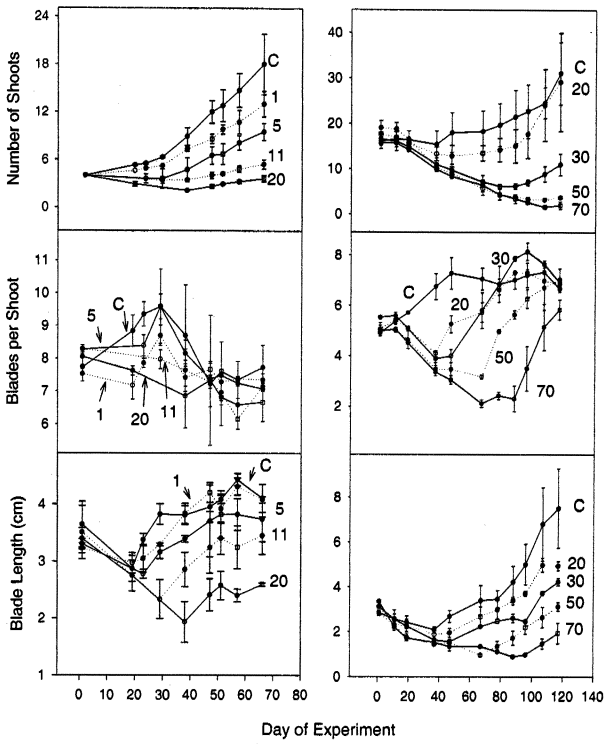

Fig. 5. Mean (n = 2 mesocosms) number of shoots, blades per shoot, and length of blades in the treatments during the two experiments. Means calculated by first averaging across tubs in a mesocosm and then averaging the values (n = 2) for the mesocosms. C = control, numbers refer to exposure treatment (1, 5, 11, 20, 30, 50, 70 d). Open circles = end of exposure, open squares = end of 1-mo recovery. Error bars are ± 1 standard error.

day of the experiment. At the end of the experiment blade length was similar in the control, 1-d, and 5-d treatments, but all were significantly longer than in the 20-d treatment (LSD test). Blades in the 11-d treatment were of intermediate length (Fig. 5).

Repeated measures analysis of variance (treatment × sample date) showed that by the end of the long-term experiment, numbers of shoots and blades in the 20-d and 30-d treatments were statistically similar to the control (LSD test, $p > 0.05$) and except for shoots in the 30-d treatment, had recovered to initial levels (Fig. 5). Compared to the control, the 50-d and 70-d treatments had significantly (LSD test, $p < 0.05$) fewer blades and shoots and had not recovered to initial levels (Fig. 5). The number of blades per shoot in all exposure treatments recovered to initial levels within a month following exposure. At the end of the experiment there were no statistical differences between treatments.

In contrast to the ANOVA comparing treatments at three times, the repeated measures ANOVA using all sample dates detected significant treatment × time effects for blade length in the long-term experiment (Fig. 5). In all exposure treatments, exposure to 18‰ resulted in a decrease in average blade length relative to initial values (LSD, $p < 0.05$). Blade length returned to initial levels after 1 mo in the 20, 30, and 50-d exposure treatments. After 1 mo, blades in the 70-d treatment were still significantly (LSD, $p < 0.05$) shorter than at the beginning of the experiment. At the end of the experiment, significant differences between treatments persisted (nested ANOVA, $p < 0.05$) and blade length decreased with increasing duration of exposure (Pearson's $r = -0.893$, $n = 10$, $p < 0.05$, Fig. 5).

Rates of Recovery

Data from the short-term experiment were sufficient to calculate and compare exponential growth rates from all treatments for days 38–66 of the experiment (Fig. 5). Exponential growth coefficients ranged from 0.017 to 0.026 d^{-1} (Table 5) and analysis of covariance detected no statistical

TABLE 5. Exponential growth coefficients for shoots, r (SE), calculated for each treatment in the two experiments. Also given are the r^2 for the regression, the time period (days of experiment) used to calculate r, and n the number of observations. * indicates statistical significance at $p < 0.05$.

Treatment	r	r^2	n	Days
Short-term experiment				
Control	0.024 (0.002)	0.949*	10	38–66
1 day	0.020 (0.005)	0.685*	10	38–66
5 day	0.026 (0.006)	0.674*	10	38–66
11 day	0.017 (0.001)	0.953*	10	38–66
20 day	0.019 (0.004)	0.766*	10	38–66
Long-term experiment				
Control	0.013 (0.003)	0.742*	8	88–117
20 day	0.022 (0.003)	0.877*	8	88–117
30 day	0.019 (0.003)	0.876*	8	88–117
50 day	0.014 (0.003)	0.934*	4	107–117

differences between them (F-test for equal slopes $p > 0.20$).

In the long-term experiment, exponential growth rates were calculated for days 88 to 117 for the control, 20-d and 30-d treatments, and for days 107 to 117 for the 50-d treatment. Exponential growth coefficients ranged from 0.013 to 0.22 d^{-1} (Table 5) and analysis of covariance detected no statistical differences between them (F-test for equal slopes, $p > 0.40$).

Discussion

While *V. americana* is clearly salt tolerant, estimates of tolerance limits vary in the literature. Cessation of growth has been reported to occur at 8.4‰ (Bourn 1932, 1934), 6.66‰ (Haller et al. 1974), and 15‰ (Doering et al. 1999). Laboratory experiments led Haller et al. (1974) to conclude that death occurred at 13.3‰, while field observations suggested that death occurred at salinities greater than about 15‰ (Kraemer et al. 1999). After five weeks of exposure Twilley and Barko (1990) found no effect of salinity on growth or mortality in the range of 0‰ to 12‰. Differences between studies may be due to differences in overall methodology, to varying methods of exposure (Twilley and Barko 1990), or to real differences in salinity tolerance between populations (Doering et al. 1999).

Results presented here show that mortality of *V. americana* from the Caloosahatchee occurs at 18‰, but the duration of exposure is important. Statistically significant loss of blades and/or shoots occurred after 20 or more days of exposure. This lethal limit determined in the laboratory agrees with transplant experiments conducted in the Caloosahatchee by Kraemer et al. (1999). At one downstream site, complete mortality occurred after 2 to 4 weeks of exposure to salinities that were increasing from 15‰ to 22‰ (Kraemer et al. 1999).

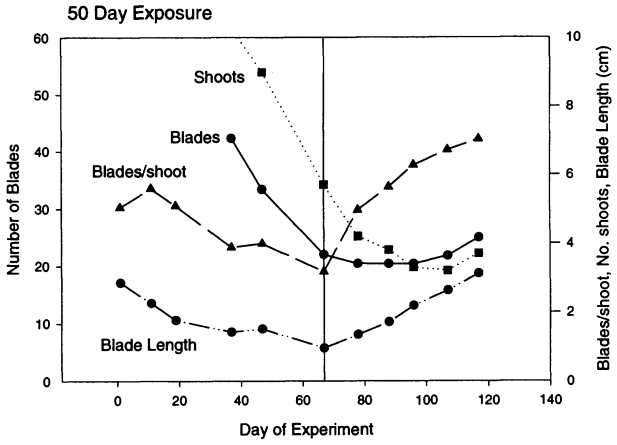

Fig. 6. Time course of recovery for average number of shoots, blades, blades per shoot, and blade length in the 50-d exposure treatment. The vertical line indicates the end of exposure to 18‰.

In our experiments, the degree of mortality was proportional to the duration of exposure to 18‰. The regression equation (Fig. 4) indicates that an exposure of 31 d would cause a 50% loss of shoots, with 10% remaining after 95 d. The relationship between mortality and duration of exposure at higher salinities remains to be investigated.

While we observed mortality at 18‰, viable plants still persisted after 70 d, suggesting that *V. americana* shoots can survive most salinity intrusions in the upper Caloosahatchee estuary. Our experiments examined the response of *V. americana* to single exposures of varying duration. How *V. americana* might respond to multiple exposures of different strength and frequency remains to be investigated.

We are unaware of any previous studies examining the recovery of estuarine submerged aquatic vegetation from salinity stress. The regression equations in Fig. 4 quantify the effects of duration of exposure to 18‰ on the ability of plants to recover following return to favorable conditions. Plants exposed to 18‰ for 30 d could achieve a 50% recovery of lost blades and shoots during the following month. Plants exposed to 18‰ for 15 d could fully recover to initial levels within a month at 3‰.

Our results indicate that recovery is a two-stage process characterized by an initial allocation of energy to production and elongation of blades on individual shoots followed by the clonal production of new shoots. Qualitative, temporal changes in plant attributes support this scenario and such changes in the 50-d treatment are illustrative (Fig. 6). Immediately following exposure, both blade length and the number of blades per shoot increased, even as the total number of blades and

shoots continued to decline. The number of blades began to increase next due to the continued increase in number of blades per shoot and a reduction in the loss of shoots. Finally new shoots were produced.

The two-stage recovery scenario also is supported by the order in which plant attributes recovered after exposure in the long-term experiment. After 1 mo, blades per shoot had recovered in all treatments and blade length in all but the 70-d treatment, although number of shoots did not recover in any treatment after a month. By the end of the experiment only the 20-d treatment, with the longest time to recover, attained initial numbers of shoots.

Salinity intrusions have the potential to affect both the habitat value and the population dynamics of *V. americana* beds in the upper Caloosahatchee estuary. The abundance of birds and fish associated with submerged grass beds has been positively correlated with the abundance or biomass of grass (Kemp et al. 1984; Lubbers et al. 1990). Salinity intrusions of long duration (20–70 d) will cause mortality and reduce density of *V. americana* directly. Exposures of shorter duration (1–11 d) are less likely to cause mortality but retard growth relative to controls for a month or more. Short-term intrusions may reduce density indirectly by retarding the accumulation of grass.

The life cycle of northern and southern populations of *V. americana* differ (Dawes and Lawrence 1989). Northern populations over-winter as a dormant winter bud, buried in the sediment, and aboveground biomass disintegrates (Titus and Hoover 1991). The production of winter buds at the end of the growing season and the sprouting of leaves from these buds in the spring determine the vegetative persistence of *V. americana* (Korschgen et al. 1997). While we have observed *V. americana* plants from the Caloosahatchee to flower both in the field and laboratory, we have not observed winter bud formation in either place. The vegetative persistence of *V. americana* in the Caloosahatchee may depend largely on the survival of rosettes during the winter when saltwater intrusions are likely.

Assuming ample supplies of light and nutrients and recovery at rates equivalent to the 50-d treatment, our results indicate that a 70-d intrusion of 18‰ is at the limit of what might be tolerated without a net population reduction during the winter dry season. Such an intrusion would cause an 80% reduction in shoot density (Fig. 4). Assuming that exponential growth ($r = 0.014\ d^{-1}$, Table 5) begins as soon as the intrusion ends, this loss would be recovered in 115 d. The intrusion and recovery from it (70 + 115 = 185 d) would occupy the entire winter dry season (180 d).

A resource-based approach is being used to establish a minimum freshwater discharge to the estuary at S-79 (Chamberlain and Doering 1998). One criterion employed in the process is the amount of freshwater required to sustain beds of *V. americana* as habitat in the upper estuary. Experiments such as those reported here can help identify not only the quantity of water required but also the temporal characteristics of freshwater delivery.

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