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EFFECT OF SALINITY ON THE GROWTH OF *VALLISNERIA AMERICANA* MICHX. FROM THE CALOOSAHATCHEE ESTUARY, FLORIDA

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ABSTRACT: *The effects of salinity on the growth and survival of wild celery,* Vallisneria americana *Michx., from the Caloosahatchee Estuary (southwest Florida, USA), were examined experimentally using indoor mesocosms. Plants were exposed to 5 constant salinity treatments (0‰, 3‰, 9‰, 12‰, 15‰) for 43 days. Two factors (position in the estuary, season) which might lead to different exposure regimes and hence different salinity tolerances were also examined. Two independent experiments were conducted: one during the winter dry season and one during the summer wet season. In both seasons, plants were collected from two sites, one 5.5 km upstream of the other. The nutrient and chlorophyll concentrations in the plant tissue were analyzed at both sites. Salinity effects did not vary as a function of site, however, plants grown in sediment from the more upstream site had a higher concentration of phosphorus in blades. There was no seasonal effect on salinity tolerance limits. In both seasons, plants survived exposure to 15‰ for 43 days and net production of blades ceased at this salinity. There were seasonal differences in the pattern of response to salinity. Growth, as measured by blade elongation and production of blades and shoots, exhibited a threshold response in the dry season, with growth higher at salinities less than or equal to 3‰ and lower at salinities greater than or equal to 9‰ (0 = 3 > 9 = 12 = 15‰). In the wet season, growth declined monotonically with increasing salinity (0 > 3 > 9 > 12 > 15‰). At salinities ≤ 9‰, growth was greater during the wet season than during the dry season. Depending on the parameter, seasonal differences disappeared at 12 or 15‰.*

BEDS of submersed aquatic angiosperms are ecologically important in shallow lotic, estuarine, and marine habitats because they provide shelter

and food for many benthic and pelagic organisms and form nursery habitat for early life stages of numerous commercially important fish and shellfish. They also enhance sediment deposition and retard erosion and resuspension (Thayer et al., 1984; Kemp et al., 1984; Killgore et al., 1989; Carter et al., 1988; Lubbers et al., 1990).

No species of submersed aquatic angiosperm is strictly limited to waters of estuarine salinities. Typically, 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 these freshwater species is key to understanding their occurrence, distribution, abundance, and performance in estuarine systems.

Wild celery, *Vallisneria americana* Michx., is a salt-tolerant freshwater species that often occurs in the fresh, oligohaline, and mesohaline reaches of estuaries in the Northeastern and Southeastern United States (Bourn, 1932; Lowden, 1982). It 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 and the above-ground biomass disintegrates (Titus and Hoover, 1991). In south Florida, populations do not completely die back in winter (Dawes and Lawerence, 1989). Actively growing plants may be found all year.

While there have been several determinations of the salinity tolerance of *V. americana* (Bourn, 1932; 1934; Haller et al., 1974; Twilley and Barko, 1990) estimates do not agree and there is little information concerning factors which might modify salinity tolerance. Bourn (1932;1934) investigated potential seasonal differences in salinity tolerance of plants collected from Back Bay, Virginia and found none. Twilley and Barko (1990) compared tolerances of plants (collected from the Potomac River, Virginia) grown at high (50% incident solar radiation) and low (8% incident solar radiation) light levels and found no differences.

In estuaries, salinity typically varies temporally. The effects of such variation on the salinity tolerance of salt-tolerant freshwater species have not been investigated. In subtropical South Florida, there is a prominent seasonal cycle in estuarine salinity driven by wet season (May–October)–dry season (November–April) differences in rainfall and runoff. Plants growing at the end of the wet season may have experienced an antecedent exposure to lower salinity than those surviving at the end of the dry season when salinities are higher. Estuaries are also typified by spatial variation in salinity, sediments and other environmental factors. Salinity tolerance might vary spatially owing to different salinity exposure or other differences between sites. For example, site differences in growth and chemical composition of *V. americana* have often been attributed to contrasting sediment types (Titus and Stephens, 1983; Rybicki and Carter, 1986; Rogers et al., 1995). Here, we examine the salinity tolerance of *V. americana* collected

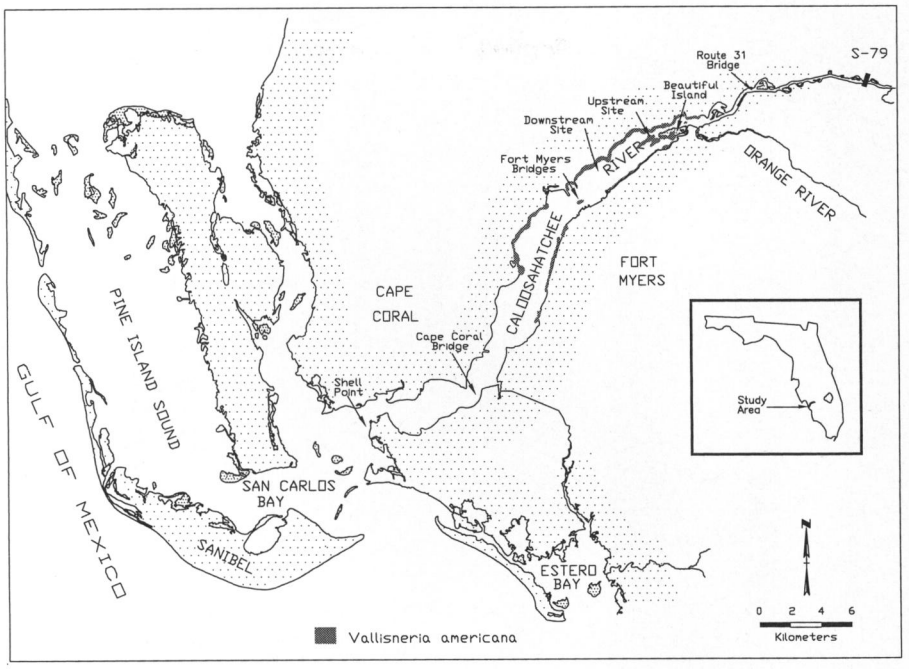
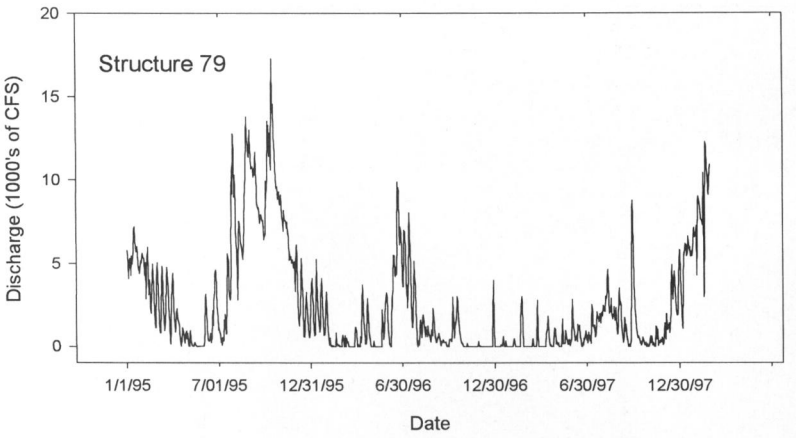


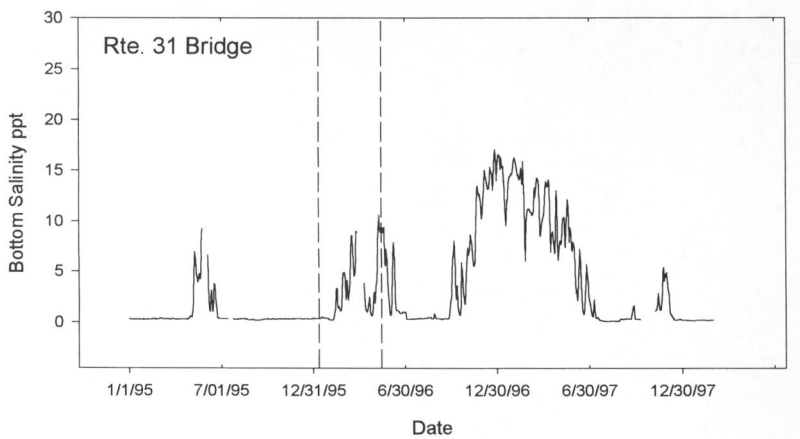
FIG. 1. The distribution of *Vallisneria americana* in the Caloosahatchee Estuary. Also shown are the sites from which *Vallisneria* was collected and the location of salinity recorders at the Route 31 and Ft. Myers bridges maintained by the South Florida Water Management District.

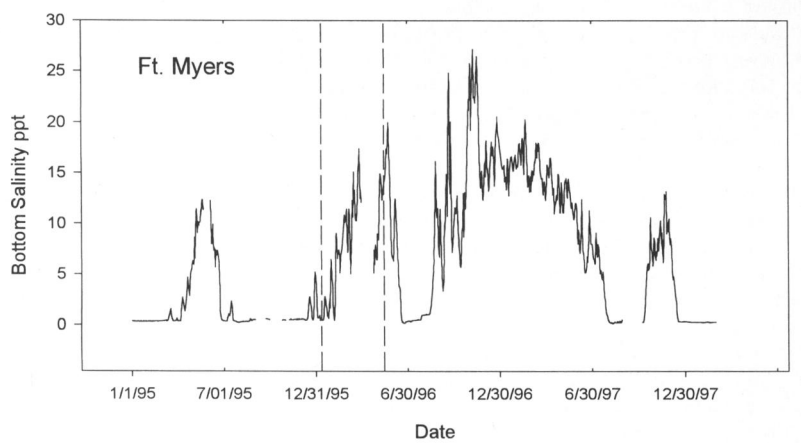
from the Caloosahatchee Estuary in South Florida and consider effects of season and position in the estuary.

STUDY AREA—The Caloosahatchee Estuary is located on the Southwest Coast of Florida, USA (Fig. 1). The major source of freshwater to the estuary is the Caloosahatchee River which runs 65 km from Lake Okeechobee to 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 Ft. Myers bridges, especially around Beautiful Island (Fig. 1, Hoffacker, 1994). The beds may be important habitat for fish and shellfish as described previously and also may comprise a food source for a resident population of West Indian manatees (*Trichechus manatus*). *V. americana* can be a major dietary component of the manatee and is sometimes preferred over other submersed aquatic vegetation (Packard, 1981). Anecdotal and qualitative observations indicate that in some years beds may be extensive and quite lush in both seasons, while in other years, only sparse populations of small plants may be found.

The magnitude of freshwater discharge at S-79 varies temporally (Fig. 2). As data from continuous salinity recorders in the downstream estuary show, this variability causes salinity upstream of the Ft. Myers bridges to vary on several time scales (Fig. 2). During the winter dry season, upstream intrusion of seawater causes salinity to exceed the tolerance limits for *V. americana* reported in the literature (up to 12‰; Twilley and Barko, 1990). In the summer wet season, discharge can turn the system entirely fresh. The record also shows considerable variation in the magnitude and duration of these seasonal intrusions of saltwater. On shorter time scales (3–7 days) salinity can change rapidly. Rates of 1‰/day at Bridge 31 and 2‰/day at







the Ft. Myers bridges are common in the record. The record also shows that during the dry season, salinity varies spatially. This study was undertaken 1) to determine the influence of salinity on the growth and survival of V. americana 2) to better understand the effects of freshwater discharge and attendant salinity variation on the distribution and abundance of *V. americana* and 3) to help identify the level of freshwater discharge at S-79 required to maintain *V. americana* upstream of the Ft. Myers bridges.

MATERIALS AND METHODS—A mesocosm approach was used to examine the influence of salinity on the growth and survival of *V. americana.* Plants were grown in ten cylindrical tanks (1.3 m in diameter × 1 m deep) filled with water to a depth of 60 cm (volume = 800 l). The tanks were located indoors at an aquarium facility at the Gumbo Limbo Nature Center in Boca Raton, FL. A 1000 Watt metal halide lamp, kept on a L:D photoperiod of 12:12 h, supplied light to each tank. A given salinity was maintained by mixing appropriate volumes of fresh and salt water (total volume = 114 l) from each of two head tanks located above each mesocosm. Head tanks were alternately filled and emptied into the mesocosms using solenoid valves controlled by timers. Thus, water was delivered to the mesocosms in a series of 114-liter pulses. Water in the mesocosms was replaced 3 times daily. Salt water was pumped from the Atlantic Ocean. Tap water, passed through a series of activated charcoal towers and filters (20 micron pore size) to remove chlorine, was used as a source of fresh water. Seasonal variation in salinity tolerance was examined by conducting two experiments: one during the dry season (Nov.–April) and one during the wet season (May–Oct.). Similar methods, summarized below, were employed during each experiment.

Collection of plants—To examine spatial variation in salinity tolerance, *V. americana* and associated sediments were collected from each of two locations in the Caloosahatchee Estuary (Fig. 1). The downstream site was located near North Fort Myers (Lat: 26° 40′ 0.92″ N, Long: 81° 52′ 20.77″ W). The other site was approximately 5.5 km upstream. Plastic, rectangular tubs (14 cm H × 24 cm L × 15 cm W) were filled with sediment in the field. Sediment was collected with a shovel and passed through a 0.25 cm^2 mesh screen, to remove shells, pebbles, and large infauna. Plants were collected as plugs from cores taken with a post hole digger. Each plug consisted of one rosette and its root material surrounded by 30–40 ml of sediment from the site of collection. Plant plugs were placed in ice chests, covered with water from the site, and transported back to the laboratory on the day of their collection. The dry season collection occurred on 11 January, 1996, before the major seasonal intrusion of salt water (Fig. 2). The wet season collection occurred on 15 May, 1996, before summer runoff washed salt from the system (Fig. 2).

Salinity experiments—Upon return to the laboratory, plants and sediments were allotted to 4 treatment combinations and distributed to the mesocosms as follows: upstream plants in upstream sediments (2 tubs/mesocosm), downstream plants in downstream sediment (2 tubs/mesocosm), upstream plants in downstream sediment (1 tub/mesocosm), and downstream plants in upstream sediments (1 tub/mesocosm). Four plant-plugs, including 30–40 ml of sediment from the site of collection, were planted in each tub.

After a six-week acclimation period in fresh water, shoots produced since initial planting were removed to standardize initial conditions. To reflect conditions in the field, salinities were gradually raised (1.5‰/day) to final treatment salinities of (0‰, 3‰, 9‰, 12‰ and 15‰). Two mesocosms were randomly assigned to each salinity treatment. Plants were held at treat-

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FIG. 2. Daily average freshwater discharge (cfs) at the Franklin Lock and Dam (S-79) and salinity at the Route 31 and Ft. Myers bridges from records maintained by the South Florida Water Management District. Dashed lines mark the days upon which plants were collected.

ment salinities for 43 days. The dry season experiment commenced on March 1, 1996, and the wet season experiment on July 11, 1996. Tanks were scrubbed as necessary to remove wall growth. Once a week, epiphytic growth was gently removed by hand from plant blades. Salinity and temperature were monitored daily with a YSI 600XL data sonde. PAR was checked weekly with a LICOR spherical quantum sensor and data logger and adjusted to 475–525 μmol photons/m^2/sec at the bottom of the tank by raising or lowering the lamp. Water samples were analyzed weekly for dissolved inorganic nitrogen and phosphorus on an Alpkem Auto Analyzer (APHA, 1985; SFWMD, 1994).

Plant response variables—The following non-destructive measurements were made weekly during each experiment: number of shoots per tub, number of blades per shoot, and blade length of 30 randomly selected blades per tub. In addition, one new blade in each tub was marked with a piece of thread sewn through the blade. Lengths were measured at the time of marking and after one week.

The oxygen metabolism of *V. americana* blades was measured at the end of the wet and dry season experiments. At least two blades were removed at the sediment surface from each tub of the site treatments (excluding cross plants). These were placed in clear and opaque 300 ml BOD bottles and returned to the bottom of each tank for a 15 min acclimation period. Initial dissolved oxygen (DO) was measured in each bottle with an Orbisphere model 2607 oxygen meter equipped with a miniature sensing head (model 2112). Light and dark incubations lasted approximately 60 and 120 minutes, respectively, after which final DO was measured in each bottle. Blades were removed from each bottle, dried at 80 °C, and weighed for dry mass. DO evolved during the incubations was normalized per unit dry mass. Because dark bottle results were variable and often showed oxygen evolution, only net primary production data, calculated from the light bottles, were analyzed.

At the end of each experiment, plants were harvested from each tub. Three 0.5 cm^2 sections of blade from each tub were frozen for chlorophyll *a* analysis. Blades were dried to constant weight at 80 °C and ground to a fine powder for determination of nitrogen and phosphorus content.

Samples for chlorophyll *a* analysis were ground in 90% acetone and extracted at 0 °C for three hours. Chlorophyll *a* concentration was determined spectrophotometrically on a Perkin Elmer UV/VIS Lambda 12 spectrophotomter (APHA, 1985; SFWMD, 1994). Tissue nitrogen was determined by elemental analysis on a Fissons CNS Analyzer Series 2 against a BBOT ($C_{26}H_{26}N_2O_2S$) standard. Analytical replicability was ± 5%. Recovery of NIS reference material was 95–104% for nitrogen in peach and spinach leaves. Tissue P was analyzed by a method modified from Solorzano and Sharp (1980). Ground 50 mg samples were ashed (550 °C) and hydrolyzed at 80 °C with 0.025 *N* HCl. Hydrolyzed samples were diluted 1:100 with distilled water and analyzed for soluble reactive phosphorus on an Alpkem Auto Analyzer. Analytical replicability was ± 5%, and recovery of P from NIS reference material was 88–104% for both peach and spinach leaves.

Statistical analysis—The experimental design included four factors: season (wet or dry), site (upstream plants and sediment or downstream plants and sediment), salinity (0, 3, 9, 12, 15‰) and replicate (the two mesocosms at each salinity). The cross-planted tubs (upstream plants in downstream sediments and downstream plants in upstream sediments) were included to help explain differences between sites only if these were statistically detected. Cross-planted tubs were omitted from the initial analysis of the data.

To avoid pseudoreplication, data taken on a given day in each mesocosm were averaged across the two tubs from each site. This procedure yielded one observation from each of the two mesocosms assigned to each site × salinity combination. A four factor ANOVA, with interaction, was used to statistically evaluate the data taken at the end (Day 43) of the experiment. For non-destructive measurements, data taken on Day 1 of the experiment were similarly analyzed. The replicate factor (two mesocosm tanks assigned to each salinity) was considered to be random and nested with the salinity factor. In such a design, the replicate mesocosm is

the experimental unit. Rules for determining F-tests in nested ANOVA designs are summarized in Winer (1971). Differences between main effect and interaction means were evaluated with the Student-Newman-Keuls test (Winer, 1971).

Blade elongation rates measured by marking newly formed blades with a thread were analyzed differently. The marking technique was not 100% effective for two reasons: threads often worked their way out of a leaf and at times, the narrow width of blades precluded marking in the first place. As a result during some weeks not all mesocosms contained marked leaves. To ensure a more balanced design, the mesocosm factor was dropped and data taken over the entire course of the experiment were evaluated using a 3-factor (Season, Site, Salinity) ANOVA with interaction. All statistical analyses were accomplished using SAS Version 6 (SAS, 1989).

The ANOVA approach examines the relative differences between treatments but does not quantify growth rates. Weekly measurements were used to determine net rates of blade production, shoot production, and change in average blade length. Growth was modeled using the exponential growth equation ($N_t = N_0 e^{rt}$). Again, data taken on a given day in each mesocosm were averaged across the two tubs from each site. Regressions were calculated for each salinity treatment.

RESULTS—With few exceptions, measured salinities over the course of both experiments were maintained within ±1‰ of nominal treatment salinities (Fig. 3). Temperature in the ten mesocosms varied by ± 2 °C at any one time and averaged (all ten tanks) 25.2 ± 0.8 °C during the dry season and 27.2 ± 0.9 °C during the wet season. Three-way ANOVA (season, salinity, replicate) revealed that in all salinity treatments, temperatures were higher in the wet season than in the dry season ($p<0.05$). Dissolved inorganic nitrogen (DIN) concentrations were about 10 μmol higher in the dry than the wet season (Table 1). In contrast, dissolved inorganic phosphorus (DIP) was slightly higher in the wet season than the dry season. Both DIN and DIP declined with increasing salinity.

Morphometric measurements—At the beginning (Day 1) of the two experiments, conditions were similar in each salinity treatment. Tubs in each of the mesocosms averaged about 4 shoots/tub, with 10 to 14 blades/shoot and blades 3 to 4 cm in length ($p>0.05$ for main effect of salinity, and $p>0.05$ for all interaction terms involving salinity) . There were some statistical differences associated with the site factor. At the beginning of the wet season experiment, plants from the upstream site had more blades per shoot (about 13) and longer blades (3.9 cm) than plants from the other three season × site combinations (11–12 blades/shoot, 2.9–3.1 cm in length). Because the site factor was completely crossed with the salinity factor, these site differences were independent of salinity and do not affect experimental results for salinity.

After 43 days, no site effects were detected. However, a significant interaction between season and salinity was detected ($p < 0.05$) for average blade length, number of blades/tub, and number of shoots/tub. There were differences in the response of *V. americana* to salinity in the two seasons. In the dry season, number of blades and number of shoots exhibited a threshold response with greater production at 0‰ and 3‰ than at higher salinities.

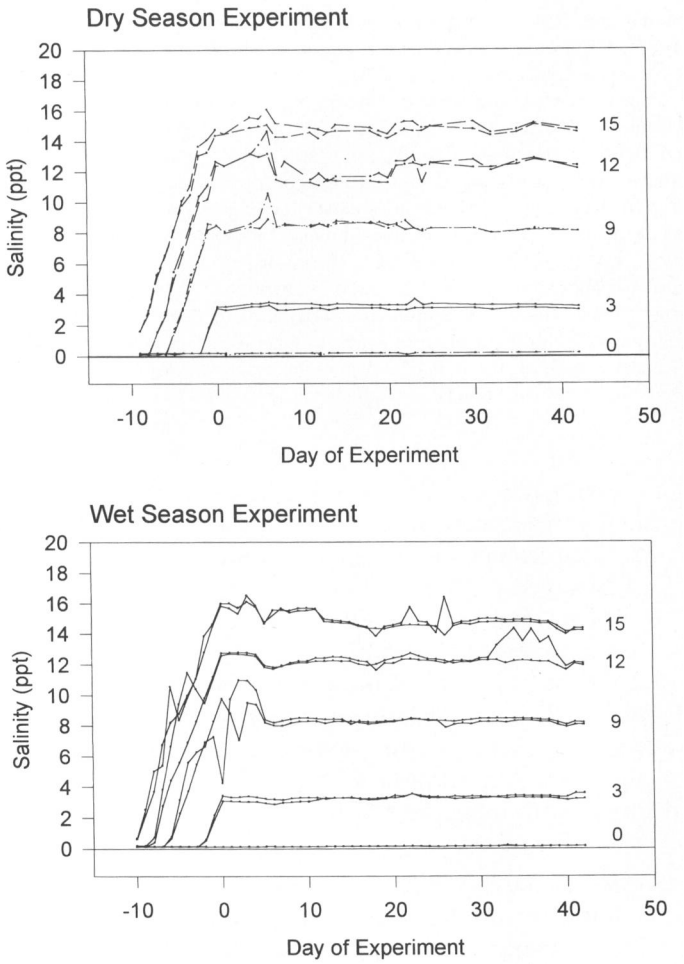

FIG. 3. Time course of salinity in the mesocosms during the two seasonal experiments.

Blade length did not vary with salinity. In the wet season, blade length, number of blades, and number of shoots all decreased with increasing salinity. The statistical differences between specific treatment means (Fig. 4) were slightly different for each parameter, but all showed the same general pattern.

Seasonal differences also varied as a function of salinity. At 0‰, 3‰, and 9‰, the number of blades and shoots produced was higher in the wet season than in the dry season and blades were longer ($p < 0.05$, Fig. 4). Seasonal differences disappeared at 12 and 15‰ (Fig. 4).

Rates of growth—Elongation rates (cm/day) of newly formed blades measured by marking with a thread also varied with salinity and season

TABLE 1. Average concentrations of dissolved inorganic nitrogen (DIN = NH_4 + NO_2 + NO_3) and dissolved inorganic phosphorus in the two mesocosms assigned to each salinity. Units are μmole/l.

	Dry season		Wet season	
Salinity	DIN	DIP	DIN	DIP
0	35.5 ± 4.5	2.6 ± 2.1	22.8 ± 4.5	3.1 ± 0.4
	37.8 ± 5.5	2.6 ± 2.1	21.2 ± 3.5	3.3 ± 0.3
3	34.1 ± 4.0	1.9 ± 1.8	21.6 ± 3.0	3.2 ± 0.4
	35.1 ± 4.0	2.5 ± 2.0	24.2 ± 2.7	3.2 ± 0.5
9	31.0 ± 3.7	2.2 ± 1.8	19.5 ± 3.2	3.2 ± 0.5
	31.4 ± 3.7	2.3 ± 1.8	20.5 ± 3.0	2.9 ± 0.3
12	30.4 ± 3.8	2.0 ± 1.6	17.2 ± 1.9	2.5 ± 0.3
	27.5 ± 3.6	2.0 ± 1.6	17.1 ± 1.8	2.5 ± 0.4
15	27.0 ± 3.1	1.8 ± 1.4	16.5 ± 4.1	2.2 ± 0.2
	22.1 ± 6.9	1.7 ± 1.4	14.9 ± 2.1	2.3 ± 0.2

(Fig. 5). During the winter dry season, there were significant differences between salinity treatments. However, elongation rates did not vary with salinity in any regular way. Blades grew as fast at 15‰ as they did at 0‰. During the wet season, growth rates declined with increasing salinity being highest in freshwater, somewhat lower at 3‰, and markedly reduced at 9, 12 and 15‰. Seasonally, wet season growth rates were higher at 0, 3, and 9‰. No seasonal differences were found at 12 or 15‰.

Significant positive net production of shoots, calculated from weekly counts, occurred at all salinities (Table 2). By contrast, net production of blades ceased at 15‰ in both seasons (Table 2). In the dry season, rates of increase in average blade length were significantly greater than zero ($p<0.05$) at all salinities. In the wet season, average length actually decreased at 15‰ (Table 2). Within each season lowest rates of shoot production, blade production, and change in blade length were found at 15‰. Highest rates occurred at 0 and 3 ‰.

Measurements of instantaneous oxygen production by excised leaves were quite variable and ANOVA revealed no significant effects of season or salinity. However, a linear regression of net primary production on salinity including all 80 data points was significant. The relationship (O_2/mg dry mass/hr = -0.002(Salinity) + 0.044, $p < 0.01$) suggested a negative effect of salinity on production and explained 20% of the total variation.

Chemical composition—The concentration of nitrogen in blades ranged from 19 to 31 mg N/gm dry wt. Concentration decreased with increasing salinity in both seasons but the pattern was significant only in the wet season (Fig. 6). Phosphorus in blades ranged from 2.3 to 5.2 mg P/gm dry wt and varied with season and salinity in a manner similar to nitrogen. N:P atomic ratios ranged from 20 to 24 in the dry season and from 14 to 19 in wet

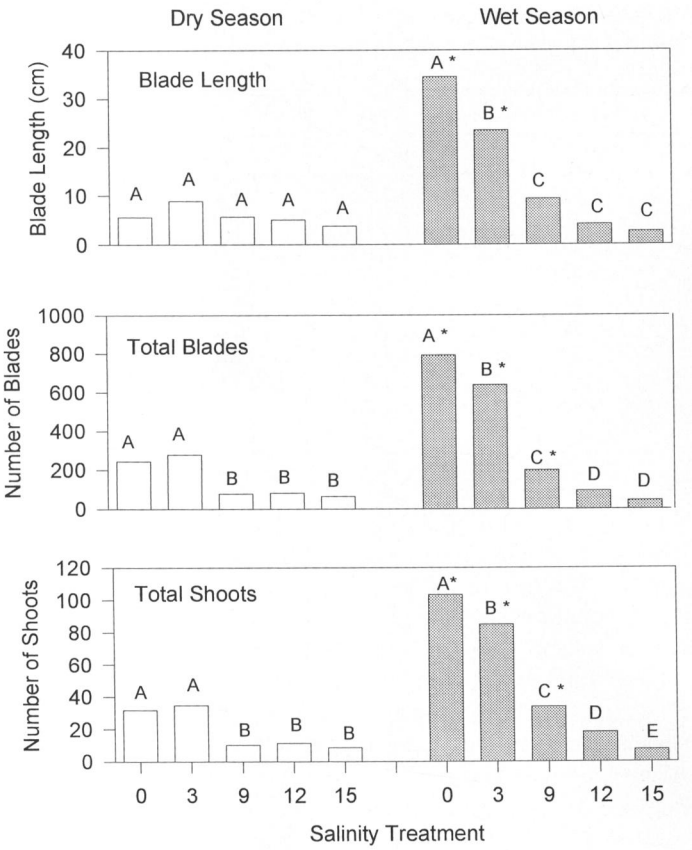

FIG. 4. Average blade length, number of blades and number of shoots per tub in the salinity treatments. Letters indicate statistical differences between salinity treatments in each season. Means with different letters are statistically different at $p<0.05$. Asterisk (*) indicates a significant difference ($p<0.05$) between seasons at a particular salinity.

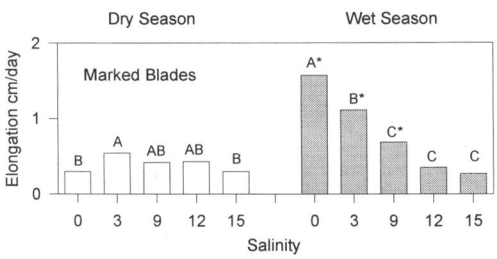

FIG. 5. Elongation rates of individual marked blades in the salinity treatments. Letters and asterisk are as in Fig. 4.

TABLE 2. Exponential growth coefficients, r, (95% CI) for total blades, total shoots and average blade length in two seasons (wet, dry) and 5 salinity treatments. ns = not statistically different from zero.

Attribute	Season	Salinity Treatment (‰)				
		0	3	9	12	15
Blades	Dry	0.046 (0.003)	0.042 (0.009)	0.013 (0.007)	0.013 (0.003)	0.009 (0.010)ns
	Wet	0.071 (0.004)	0.061 (0.004)	0.032 (0.004)	0.015 (0.006)	−0.002 (0.006)ns
Shoots	Dry	0.053 (0.003)	0.054 (0.007)	0.021 (0.008)	0.027 (0.002)	0.018 (0.013)
	Wet	0.081 (0.006)	0.071 (0.005)	0.052 (0.006)	0.036 (0.008)	0.012 (0.008)
Average length	Dry	0.010 (0.005)	0.027 (0.004)	0.015 (0.004)	0.012 (0.003)	0.006 (0.003)
	Wet	0.054 (0.003)	0.042 (0.005)	0.019 (0.003)	0.004 (0.003)	−0.006 (0.005)

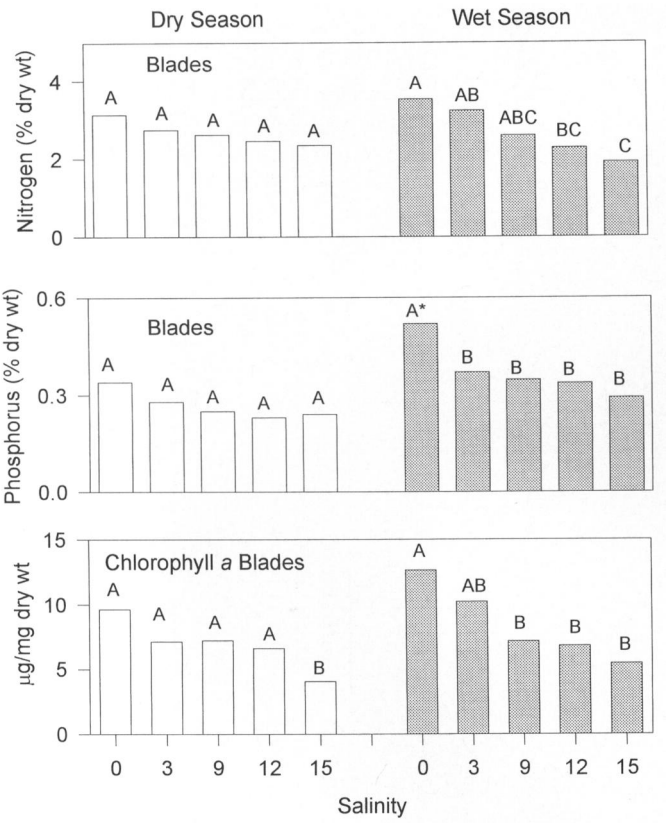

FIG. 6. Average concentration of nitrogen, phosphorus and Chlorophyll *a* in blades of *Vallisneria americana* in each salinity treatment. Letters and asterisk are as in Fig. 4.

season with no differences between salinities. The concentration of chlorophyll *a* in blades decreased with increasing salinity in both seasons.

Site effects—The chemical composition of *V. americana* was affected by position in the estuary. Overall, blades from downstream plants, growing in downstream sediments, had higher N:P ratios (atomic) than blades from upstream plants growing in upstream sediments (21.8 vs 16.9 for blades; main effect F-test, $p < 0.01$, interactions with site factor not significant). This difference was significant even if data from the two seasons were analyzed separately. The difference in N:P ratio was due to higher phosphorus concentrations in blades from the upstream site. Nitrogen concentration in blades did not differ between sites ($p > 0.05$) whereas phosphorus in blades from the upstream site (3.6 mg P/gm dry wt) was greater than in plants from the downstream site (2.8 mg P/gm dry wt; main effect F-test $p < 0.01$ in both cases, interactions with site factor not significant). These differences

were statistically significant when the data for each season were analyzed separately.

Reanalysis of the data to include the cross planted treatments as additional levels of the site factor clearly showed that the concentration of P in blades varied as a function of the sediment in which the plants were grown (main effect F-test, $p < 0.01$; interactions with site factor not significant). Results of the SNK test revealed that the treatment means fell into two groups according to the type of sediment in which plants were grown: upstream plants in upstream sediments (3.6 mg P/gm dry wt) = downstream plants in upstream sediments (3.5 mg P/gm dry wt) > downstream plants in downstream sediments (2.8 mg P/gm dry wt) = upstream plants in downstream sediment (2.8 mg P/gm dry wt). These results suggest that differences between sediments from the two sites account for the compositional differences between plants.

DISCUSSION—The ecological impacts of salinity variations are difficult to assess because other factors, such as nutrient concentration, can co-vary with salinity even under the controlled conditions used here. The mixing of ocean water and municipal fresh water in our experiment resulted in nutrient concentrations which decreased with increasing salinity (Table 1) but not to concentrations likely to have limited growth. In addition, blade tissue concentrations exceeded the levels associated with nutrient limited growth (1.3 N % dry wt and 0.13 P % dry wt) established by Gerloff and Krombholz (1966). Light also was not confounded with salinity since it was maintained at levels greater than the I_{sat} of 200 μmol photons/m^2/sec for *V. americana* (Harley and Findlay, 1994).

Salinity tolerance—Salinity tolerances of *V. americana* reported in the literature vary. Bourn (1932, 1934) examined material from Back Bay, VA. In both winter and summer experiments, growth declined with salinity and ceased at 8.4‰. Haller and co-workers (1974) collected plants from canals in Ft. Lauderdale, FL. They reported growth at 0.17‰ and 3.33‰, no growth after 4 weeks at 6.66‰ or 10‰ and death at 13.3‰ and 16.6‰. Twilley and Barko (1990) grew *V. americana,* collected from the Potomac River, VA for five weeks and found no effect of salinity on growth over the range 0‰ to 12‰. They attributed this to the method of exposure. They slowly raised (1.5–2‰/day) salinities to treatment levels over a period of days, whereas in previous studies, plants were transferred directly to treatment salinities and acutely exposed to high salinity.

V. americana from the Caloosahatchee Estuary survived 6 weeks exposure to 15‰ in both seasons: a higher salinity than has heretofore been reported. At 15‰ growth continued at a slow rate for some parameters (e.g. number of shoots) but increases in total number of blades ceased in both seasons. Additionally, average blade length declined during the summer wet season. A salinity of 15‰ is at or near the upper tolerance limit for growth

in the Caloosahatchee. Although the pattern of response differed in the two seasons (threshold in winter, steady decline in summer), the general finding of decreasing growth with increasing salinity is consistent with the results of Haller and co-workers (1974) and Bourn (1932, 1934). Like Twilley and Barko (1990), we raised the salinity slowly (1.5‰/day) to treatment levels over a period of days. Method of exposure may not explain all differences between studies.

An alternative explanation is that there are real differences in salinity tolerances among populations of *V. americana. V. americana* does exhibit substantial morphological variation in inflorescence structure and growth form (Lowden, 1982). There may also be substantial physiological and genetic variability, which could account for the reported differences in salinity tolerance summarized here. A comparative study, testing this hypothesis, has not been done.

Although there were seasonal differences in growth in the lower salinity treatments (0–9‰), we found no evidence that overall salinity tolerance limits varied seasonally. For example, the salinity at which growth ceased or at which plants could no longer survive did not vary seasonally. Despite very different exposure regimes prior to the two experiments (Fig. 2) , *V. americana* survived and, for all but one parameter (change in average blade length; Table 2) , responded similarly at 15‰ in both seasons.

As has been found in other studies of *V. americana* from Florida (Dawes and Lawrence, 1989), growth occurred in both seasons with higher growth rates in summer than in winter (Table 2). The winter 'die back', typical of more northern populations (North Carolina: Zamuda, 1976; Pennsylvania/ New York: Sullivan and Titus, 1996; Maryland: Rybicki and Carter, 1986; Wisconsin: Donnermeyer and Smart, 1985) is not complete in central and south Florida (Dawes and Lawrence, 1989) and actively growing rosettes may be found all year.

The growth of *V. americana* is influenced by temperature (Zamuda, 1976; Biernaki et al., 1997). Relatively warm temperatures could account for winter growth in southern locations. Even though the seasonal difference in temperature that we observed was small (about 2 °C), this in part may account for the higher wet season growth in our experiment. Our results indicate that increasing salinity decreases the seasonal difference in growth until it disappears at 12 to 15‰.

Position in the estuary (upstream vs downstream) also did not affect salinity tolerance despite the potential for different exposure regimes. Although we did not measure salinity at the two collection sites during our experiment, the data in Fig. 2 suggest a difference in salinity. In 1996 and 1997, Kraemer and co-workers (1998) measured salinity on 10 occasions during each season at the downstream collection site and at a site 100 yards south but adjacent to the upstream site. During the wet season salinity was similar at each site (paired t-test, $p > 0.05$, $n = 10$). During the dry season the salinity difference was only about 2 ‰ (13.5‰ downstream vs 11.7‰

upstream, paired t-test $p < 0.05$, $n = 10$) and probably too small to cause any difference in tolerance. While differences between sediments from the two sites may have affected *V. americana*'s chemical composition, no evidence for a site or sediment effect on salinity tolerance was detected.

Chemical composition—The concentration of nitrogen in blade tissue, and to a lesser extent phosphorus, declined with increasing salinity. For nitrogen, this pattern conflicts with that reported by Twilley and Barko (1990), who found an increase in nitrogen concentration with increasing salinity. Water column concentrations in Twilley and Barko's experiment were very low (maximum 1.6 μmol/l) and did not vary with salinity. They interpreted the increase in blade nitrogen as an osmoregulatory response. Our water column concentrations were substantially higher and mean concentrations declined with salinity ($r = -0.932$ and -0.907 for dry and wet seasons respectively, $n = 10$ and $p < 0.001$ in each case). Thus, the decrease in blade tissue nitrogen with increasing salinity ($r = -0.872$ and -0.985, $n = 10$ and $p < 0.01$ in each case) may have been a response to decreasing water column concentrations. Kemp and co-workers (1984) reported a similar effect in *Potamogeton perfoliatus.* Blade nitrogen increased in response to increasing nutrient loading.

The decline in the concentration of chlorophyll *a* in blades with increasing salinity suggests that plants growing at high salinity have a reduced capacity to harvest light for photosynthesis. This may in part explain the progressively slower growth as salinity increased and the negative correlation between net oxygen production and salinity that we observed. Twilley and Barko (1990) detected no effect of salinity on concentration of chlorophyll *a* or on growth of plants.

Effects of site on the growth, chemical composition, and survival of *V. americana* have been observed previously. Although the possible reasons for such differences are many, they have often been attributed to contrasting sediment types (Titus and Stephens, 1983; Rybicki and Carter, 1986; Rogers et al., 1995). Although we found no difference in the ability of sediments from two sites in the Caloosahatchee to support growth of *V. americana,* sediment significantly influenced the concentration of P in blades. *V. americana* obtains the majority of its P from the sediments (Carignan and Kalff, 1980), and the compositional difference that we observed perhaps reflected a difference in the mobility or concentration of P in the two sediment types.

CONCLUSIONS—Our results indicate that salinity is an important factor regulating the growth of *V. americana* in the Caloosahatchee Estuary. *V. americana* survived salinities up to 15‰, a value higher than previously reported in the literature. Net growth ceased or was very slow at 15‰ and this salinity is at or near the upper tolerance limit for growth of *V. americana* in the Caloosahatchee.

We found no evidence that position in the estuary or differences in

exposure to salinity caused by seasonal variation in freshwater discharge affected salinity tolerance. From a management perspective, the results suggest that the determination of a freshwater discharge commensurate with a suitable salinity for growth of *V. americana* in the upper Caloosahatchee need not account for a seasonally or spatially varying salinity tolerance. The growth response to increasing salinity did vary seasonally, with a threshold response in the winter and a monotonic decrease in the summer. This seasonal difference in pattern may be due to seasonal differences in growth rate. Since growth was slower in winter, the monotonic response we observed in summer may not have had sufficient time to develop in a 6 week winter experiment.

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