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Physiological Responses of Transplants of the Freshwater Angiosperm *Vallisneria americana* Along a Salinity Gradient in the Caloosahatchee Estuary (Southwestern Florida)

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ABSTRACT: Fluctuations in freshwater input may affect the physiology and survival of submerged aquatic vegetation (SAV) occurring in oligoaline to mesohaline estuarine regions. Controls on the distribution of the freshwater angiosperm *Vallisneria americana*, were investigated by transplanting ramets. Pots (3.8-l) containing ramets were distributed among four sites (upstream site [least saline], donor site, near downstream site, and far downstream site [most saline]) in the Caloosahatchee Estuary (Southwest Florida) during wet (May–August) and dry (October–February) seasons. During 2–4 mo of each season, physiological indicators were monitored, including photosynthesis, glutamine synthetase activity, and protein content in shoots, and carbohydrates and total nitrogen and carbon in shoot and subterranean tissues. Where the physical environment (light or salinity) was suboptimal, all physiological indices, except photosynthetic rate, showed similar stress responses, which ranged from a slow decline to a rapid drop in physiological function. Levels of soluble carbohydrates decreased in response to unfavorable conditions more rapidly than did insoluble carbohydrates. Shoot protein of *V. americana* declined prior to transplant death, suggesting that measuring protein content may provide a rapid assessment of physiological health. *V. americana* transplants at the low-salinity upstream site died during both wet and dry season experiments, likely in response to light limitation and/or partial burial by sediments. At the far downstream site, death occurred within 2–4 wk, and was attributable to elevated salinities (> ca. 15 ‰). Comparison of physiological responses with salinity and light regimes at the donor and near downstream sites suggest that light may ameliorate salinity stress. This study demonstrates that *V. americana*, nominally classed as a freshwater macrophyte, is capable of a remarkable degree of halotolerance.

Introduction

Estuarine organisms experience variations in water quality, resulting from the interaction between the tidal cycles of saltwater incursion and the amount of freshwater drainage from the surrounding watershed. The environmental requirements for the continued survival of estuarine organisms are often at odds with the drainage and water supply requirements of the surrounding human habitants. The Caloosahatchee Estuary, located on the southwest coast of Florida (Fig. 1), experiences freshwater inputs that are disconnected from the natural cycles of precipitation and runoff. Flood control canals drain the surrounding watershed and allow the rapid input of rainfall to the system. This input may be coupled with flood control discharges from Lake Okeechobee, introducing large volumes of fresh water into the Caloosahatchee Estuary. Additionally, water from the Caloosahatchee

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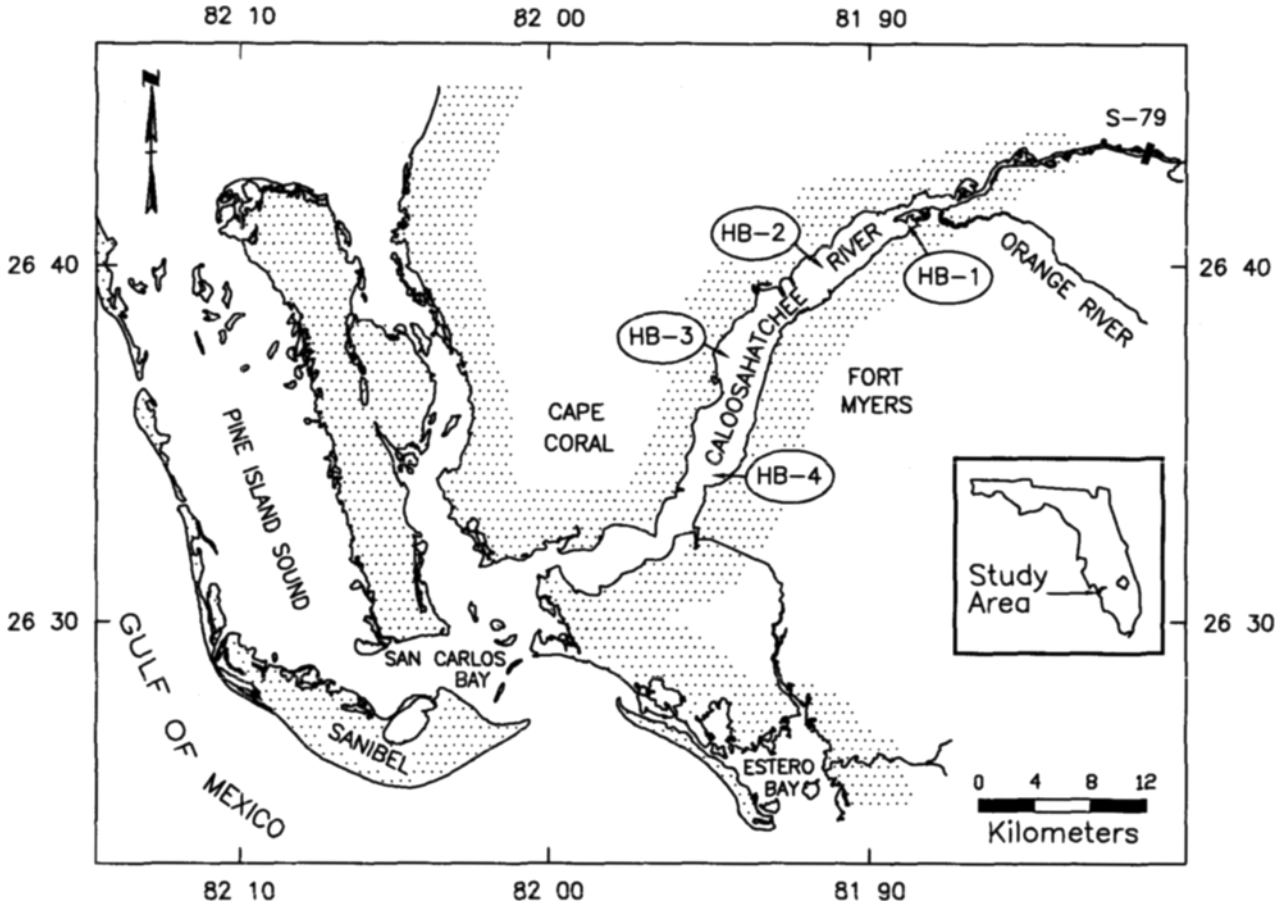

Fig. 1. Study sites in the Caloosahatchee Estuary (southwest Florida). HB-2 is the donor site from which all transplants were obtained.

Estuary watershed is diverted for human and agricultural use during periods of insufficient rainfall, allowing greater saltwater intrusion into the estuary. Together, these alterations in the natural cycles of freshwater flow into the Caloosahatchee Estuary have the potential to alter the biological nature of this estuarine system.

Submerged aquatic (vascular) vegetation (SAV) in the Caloosahatchee Estuary is an important component of this system. SAV forms the basis for the highly productive estuarine ecosystem by the de novo production of organic matter; generating physically complex habitats for a diverse group of invertebrates and fish; and providing a large substrate for the growth of epiphytic food stocks (Knox 1986). SAV at the freshwater end of the Caloosahatchee Estuary is dominated by *Vallisneria americana* (wild celery).

Variation in the physical environment of an estuary has the potential to affect the physiological health and survival of the resident SAV throughout the freshwater-saltwater gradient. Although biological differences undoubtedly exist between the oligohaline *V. americana* and euhaline seagrasses within estuaries, broad metabolic features (e.g., the production, accumulation, and use of carbohydrates) are similar in these aquatic angiosperms. Tidal action and freshwater input interact to affect the salinity and light regimes and, to a lesser extent, the temperature and nutrient levels to which SAV are exposed. Prior studies have established effects due to variation in salinity. Over the short-to-medium term, elevated salinities may cause leaf senescence (Walker and McComb 1990; Adams and Bate 1994), decreased growth (Haller et al. 1974), depressed photosynthetic rates (Kerr and Strother 1985), and increased epiphyte load (Twilly and Barko 1990). Hypersaline conditions may also cause an increase in tissue nitrogen content (Twilly and Barko 1990) and certain amino acids (Pulich 1986). The other influential environmental variable, light, is also known to affect the biology of SAV; limitations in light availability lead to reductions in growth rate and levels of stored carbohydrates (e.g., Carter and Rybicki 1990; Kraemer and Alberte 1995; Carter et al. 1996), and affect the

assimilation of inorganic nitrogen (Pregnall et al. 1987; Kraemer et al. 1997). Clearly, both carbon (C) and nitrogen (N) metabolism may be affected by fluctuations in environmental quality, which in turn may impact the SAV resident in the Caloosahatchee Estuary.

As part of a larger study to ascertain the controls on the distribution of estuarine SAV species, we transplanted *V. americana* to various sites within the Caloosahatchee Estuary to evaluate the effect of geographic location within the estuary on plant physiological health and survival. Light, salinity, and temperature were recorded at each site, and the physiology of transplanted *V. americana* was evaluated over time.

Materials and Methods

Study Location and Experimental Set-up

Vallisneria americana ramets were collected from a site in the Caloosahatchee Estuary where the macrophyte is common (site HB-2 in Fig. 1). At the donor site, plant nursery pots (ca. 3.8 l), lined with plastic bags, were partially filled with sediments that had first been sieved to remove macroscopic objects (primarily bivalves) and debris. Sediment-macrophyte plugs were then removed by hand from the bottom and placed into the pots. The *V. americana* transplants each contained at least three shoots.

Transplants were acclimated for 2 wk at the donor site. During this acclimation period, samples of transplanted and nearby untransplanted *V. americana* were collected for physiological measurements (pre-experimental samples, providing baseline data and controls for transplanting, respectively). At the conclusion of the acclimation, plants were covered and maintained damp and transported to four sites (50 pots per site; Fig. 1). Sites were chosen to provide conditions that were likely to be environmentally suboptimal (defined by location along the estuarine gradient in salinity), similar, or potentially superior to the donor site. The four sites were an upstream site (HB-1), a site lateral to the donor site (HB-2), a near downstream site (HB-3), and a far downstream site (HB-4). Resident populations of *V. americana* existed at the upstream sites (HB-1, HB-2) at the time of transplant, though nearer to shore and in shallower water at HB-1. Site HB-1 was located further upstream than the donor site, and was therefore more isolated from the incursions of saline water. Consequently, this site was potentially more hospitable to *V. americana* when freshwater influx into the Caloosahatchee Estuary was slight. Sites HB-2 and HB-3 were characterized by abundant *V. americana* growth. The far downstream site, HB-4, was exposed to higher salinities, and lacked a resident *V. americana* population. Pots containing *V. americana* were placed at each site at 60–70 cm depth (MLLW; United States National Ocean Service 1996). Therefore, differences in water quality (e.g., water-column attenuation and salinity) were attributable to position along the estuarine gradient rather than due to a difference in depth.

Sampling trips were made at 1 wk, 2 wk, and 4 wk after placement of plants for each experiment, and thereafter at monthly intervals for a total of 3–4 mo. Five pots were collected from each site during each sampling trip. The plants in each sample were separated from the sediment matrix and transported in plastic bags containing water from each collection site. Plants were stored overnight in the bags in outdoor holding tanks containing flowing water from the local estuary (i.e., the temperature should have been similar to that at the sites). The following morning at 0700 h, the samples were retrieved and the physiological analyses performed over the course of the next 3 h.

The experiment was conducted twice. The first period (May–August 1996) coincided with the wet season in south Florida, while the second (October 1996–February 1997) occurred during the dry season.

Environmental and Physiological Measurements

Environmental data were collected at each site each week during the first month of each experiment, and thereafter at biweekly intervals. Temperature and salinity were measured with a YSI model 600 XL probe. Levels of incident and submarine (10 cm, 25 cm, 50 cm, and when possible, 100 cm depths) photosynthetically active radiation were measured concurrently using a LiCor quantum meter equipped with spherical sensors. A continuous salinity and temperature sensor was also operational during both seasons at a location across the river from the donor (HB-2) site.

A series of measurements was made to assess the impact of the physical environment (i.e., position along the Caloosahatchee Estuary freshwater-saltwater gradient) on macrophyte physiology. Measurements were divided into those pertaining to carbon (C) and those to nitrogen (N) metabolism. For the former, whole shoot photosynthesis was measured in air-jacketed, 250-ml chambers. Shoots were exposed to approximately 200 $\mu mol^{-2}\ s^{-1}$ at the temperature and salinity of the collection site. This irradiance was similar to maxima at all sites during the wet season, and at HB-1 and H-2 during dry season. Noontime maxima at HB-3 and HB-4 during the dry season were ca. 330 $\mu mol\ m^{-2}\ s^{-1}$ and 620 $\mu mol\ m^{-2}\ s^{-1}$. The changes in dissolved

oxygen in the chamber were monitored for 20–25 min using an Endeco Type 1125 Pulsed D.O. system. Rates of O_2 production were standardized to fresh weight.

Samples of shoot and subterranean (roots + stolons) tissues from each pot were dried overnight at 80°C and ground to a fine powder. Levels of soluble carbohydrates were analyzed following Yemm and Willis (1954). A sample (ca. 35 mg) of dried tissue was incubated three times for 15 min in 90% methanol heated to 80°C. Extracts were dried with low heat under a ventilated hood and redissolved in a known volume of water. Aliquots of 0.60 ml of sample and sucrose standards (0–0.75 mM) were added to 1.00 ml 1% resorcinol (w/v) in 95% ethanol/concentrated HCl (1:3), mixed, and heated at 80°C for 10 min. The absorbance was read at 486 nm after cooling. The pellet remaining after the extraction of the soluble carbohydrates was incubated with 0.1 M NaOH overnight at room temperature. A 0.20 ml aliquot was added to 1.00 ml of 0.2% anthrone in dilute H_2SO_4 (1 part acid:2.5 parts H_2O) and mixed well. Tubes containing samples and sucrose standards were heated for 10 min at 100°C in a sand bath. After cooling, absorbances were read at 600 nm.

Two assays were employed to determine whether position within the Caloosahatchee Estuary had any effect on N metabolism. The in vitro glutamine synthetase (GS) activity of shoot tissue was estimated by measuring the transferase activity of the enzyme (Pregnall et al. 1987). Shoot tissue from five samples from each site were cleaned of epiphytes. Tissue was ground in ice-cold extraction buffer (50 mM imidazole, pH 7.3, 0.14% [v/v] 2-mercaptoethanol, 10 mM $MnCl_2$, 10% [v/v] glycerol, 0.03% [v/v] Tween-20, 1% [w/v] PVPP, grind ratio of ca. 10 ml g^{-1} FW). Homogenates were centrifuged to clear cell debris (10,000g, 2 min, 4°C). An aliquot of the resulting tissue extract was added to reaction cocktail (final concentrations: 470 mM imidazole, pH 7.3, 26 mM glutamine, 3 mM $MnCl_2$, 0.4 mM ADP, 20 mM arsenate, 26 mM hydroxylamine) and incubated at 35°C. Aliquots were removed from the reaction mixture at 8-min intervals, added to an equal volume of stop reagent (2 N HCl, 5% [w/v] trichloroacetic acid, 13.3% [w/v] $FeCl_3$), and quantified by spectrophotometry at 540 nm and comparison to fresh solutions of γ-glutamyl hydroxamate. Controls were run with extraction buffer substituted for tissue homogenate. Protein levels in shoot extracts were also measured (Appenroth and Augsten 1987). A 50-μL aliquot of the homogenate obtained for the GS assay was mixed with 1.00 ml of protein reagent (0.1 mg Coomassie Brilliant Blue ml^{-1} ethanol: H_3PO_4: dH_20 [volume ratio 1:2:17]). Absorbances were read at 595 nm after 10 min. Bovine serum albumen standards were used for quantification.

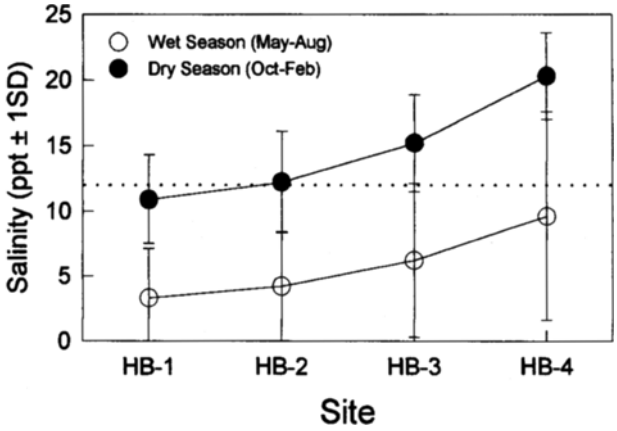

Fig. 2. Average salinities (± 1 SD) recorded at the study sites at 50-cm depth during the wet and dry seasons. The dotted line indicates the published value of the highest salinity tolerated by *Vallisneria americana* (Twilly and Barko 1990).

Dried samples were also analyzed for total tissue C and N using a Fissons Series 2 CNS analyzer. National Institute of Standards reference materials (peach leaves and BBOT [$C_{26}H_{26}N_2O_2S$]) were employed to verify measurements (95–104% recovery of C and N were recorded).

Statistical Analysis

Data were analyzed using ANOVA to examine the effect of transplant site on physiology. Tukey's multiple range test was employed in post-hoc determinations of rankings of mean values when ANOVA indicated treatment effects. Raw data were ln-transformed when sample variances were determined to be unequal using an F-test.

Results

Physical Environment

Salinity varied both among the *V. americana* study sites (along the estuarine gradient), and between the wet and dry seasons (Fig. 2). Average salinities measured over the course of the two experiments were 8–11‰ higher during the dry season than during the wet, a statistically significant difference ($F = 62.1$, $p < 0.001$). Average dry season salinity was generally higher than the highest published tolerance for *V. americana* of 12‰ (indicated by dotted line; Twilly and Barko 1990).

There were marked differences in salinity among the sites within each season (Fig. 3). During the wet season experiment, relatively little fresh water was discharged into the Caloosahatchee Estuary before ca. June 10 (1996). Salinities were elevated even at site HB-1, located ca. 30 km upstream from Shell Point. Fresh water was released

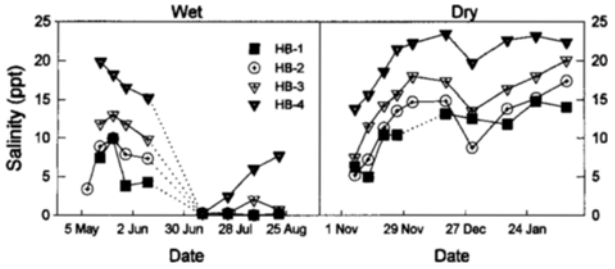

Fig. 3. Salinity time courses recorded at each study site during the wet and dry seasons. Dotted lines indicate missing intervening point

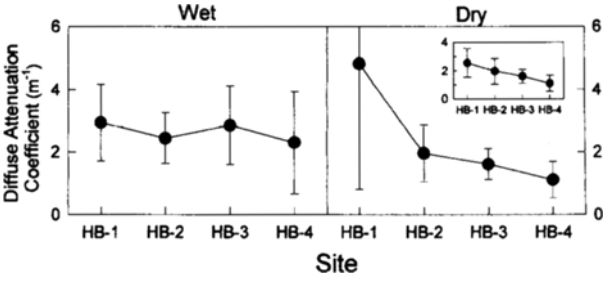

Fig. 5. Average water-column attenuation coefficients (± 1 SD) recorded at each site during the wet and dry seasons. The inset presents the same relationship, except that the two anomalously high points were excluded from the average for site HB-1.

into the Caloosahatchee Estuary in a series of pulses that began June 10 and continued through July 28 (data not shown), and salinities dropped sharply and remained low for more than 2 mo at all but the far downstream site (HB-4).

During the dry season, the initial increase in salinity was followed by a period of minimal fluctuation (Fig. 3). Sites HB-1 and HB-2 experienced salinities that were significantly lower than those at site HB-4 ($F = 8.59$, $p < 0.001$), with site HB-3 similar to all other sites. A continuous record of salinity, obtained near site HB-2 during the wet and dry seasons, showed swings of up to 8‰ over 1 wk, with a daily change of as much as 5‰ (Fig. 4).

Average water clarity, defined by the water-column attenuation coefficient, did not vary significantly during the wet season along the portion of the Caloosahatchee Estuary that encompassed the four sites (Fig. 5). During the dry season, however, the water clarity decreased with increasing distance from the Caloosahatchee Estuary mouth at the Gulf of Mexico. There were two episodes of anomalously high water-column attenuation ($k = 11.6$, 9.3 m^{-1}) at HB-1 during the dry season, contributing to the large error bar in the main graph. The two episodes probably resulted from sediment resuspension by wind-driven turbulence. Even with these two observations removed, the attenuation coefficient at site HB-1 was elevated compared with the downstream sites (Fig. 5 inset; ANOVA $F = 7.36$, $p = 0.001$; HB-1 > [HB-3, HB-4], HB-2 = [HB-1, HB-3, HB-4]). Over the course of the wet season experiment, water-column attenuation coefficients appeared generally more variable than those measured during the dry season (with the exception of site HB-1; Fig. 6). The water-column attenuation coefficients were elevated in mid to late July and this corresponds with the influx of fresh water evident in Fig. 3. Temperature varied only slightly, and there was no significant difference among sites ($F = 0.88$, $p \gg 0.05$; Fig. 7).

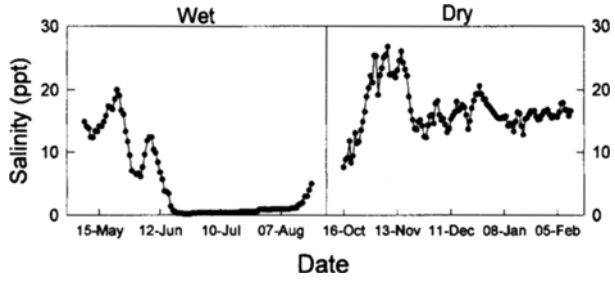

Fig. 4. Daily measurements of salinity at Fort Myers Municipal Marina, ca. 1 km downstream and across the river from site HB-2.

Field Observations of Plant Health and Survival

The *V. americana* transplants at sites HB-2 and HB-3 survived the 12-wk wet season experiment. The morphology and general appearance of the transplants at these two sites remained similar. During the second week after placement, all transplants at site HB-4 died. Death of subterranean tissue appeared to have preceded death of the shoot tissue; by the end of the first week after placement, the subterranean tissues of the HB-4 plants were brown to black, while shoots remained green in color. Transplants placed at site HB-1 site lost blades during the wet season experiment, and shoots decreased in size prior to the death of the transplants (4–8 wk after placement).

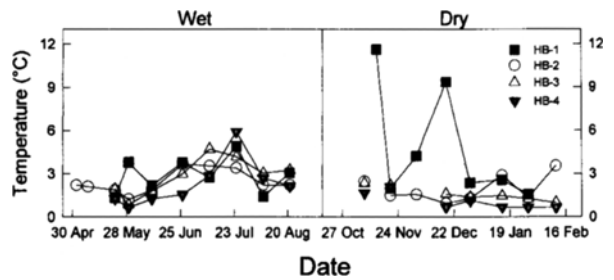

Fig. 6. Time courses of attenuation coefficient recorded at each study site during the wet and dry seasons.

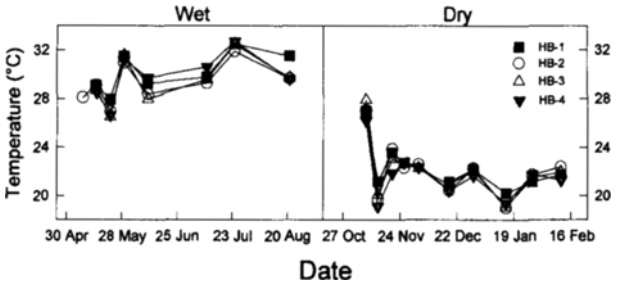

Fig. 7. Water temperatures at 50-cm depth measured at the study sites during the wet and dry seasons.

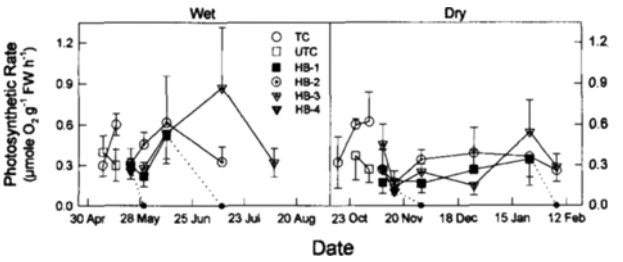

Fig. 8. Photosynthetic rates of whole shoots of *Vallisneria americana* collected at the four study sites during wet and dry seasons. Rates were measured at the temperature and salinity of the collection site. UTC = untransplanted, wild control; TC = transplanted control. Transplants were moved to experimental sites on the day the last UTC, TC samples were calculated. Dotted line leading to small, filled circles indicates the plants were dead at the time of collection.

Over the course of the dry season experiment, transplants at sites HB-2 and HB-3 lost their large summer blades, regressing in winter to small, though still growing, shoots. The formation of tubers was not observed. By the end of the dry season experiment, *V. americana* transplants maintained at site HB-3 had been heavily epiphytized by a green macroalga (cf. *Enteromorpha* sp). By the end of the first week after placement at site HB-4, the subterranean tissues of the *V. americana* transplants were brown, but the plants survived until sometime between 2 wk and 4 wk after placement. In early December (1996), the transplants at site HB-1 were found partially buried by a 2-cm layer of fine, dark sediments overlying the sandy, gray sediments characteristic of the donor site. HB-1 transplants died during the fourth month after placement at the site.

Carbon-based Physiological Indices

Prior to plant death, the rate of whole shoot photosynthesis was not affected by site during either the wet or dry season (Fig. 8). That is, a visible decrease in the photosynthetic rate of *V. americana* shoots was not visible at either site HB-1 or HB-4, sites at which plant death occurred.

The allocation of fixed carbon was affected by the treatments. Little seasonal change in soluble carbohydrates was observed in shoot and subterranean tissues of *V. americana* collected at sites HB-2 and HB-3 during the wet season (Fig. 9). At site HB-1 the levels of soluble carbohydrates in both shoots and subterranean tissues declined gradually prior to death. By the end of the first week during the wet season experiment, a significant effect of the geographic location within the estuary on the levels of soluble carbohydrates in subterranean tissues was found ($F = 8.56$, $p = 0.003$; HB-4 < [HB-2, HB-3, HB-1], HB-1 = HB-4). Soluble carbohydrate levels in shoot tissue of plants at HB-4 ranked lowest before transplant death at the end of the second week.

During the dry season experiment, soluble carbohydrate levels were similar in shoot tissues collected from sites HB-2 and HB-3 (Fig. 9). Tissue carbohydrate levels had begun to increase sharply by early February. Transplants at HB-1 did not display the late-winter accumulation of carbohydrate reserves seen in transplants at the donor (HB-2) and downstream (HB-3) sites, but reserves remained constant until death of the transplants. By the end of the first week, significant effects of the experimental site on the levels of soluble carbohydrates in the shoot ($F = 8.66$, $p = 0.001$; HB-4 < [HB-1, HB-2, HB-3]) and subterranean tissues ($F = 39.5$, $p < 0.001$; HB-4 < [HB-1, HB-2, HB-3], HB-1 > [HB-3, HB-2]) were evident.

During both seasons, the level of soluble carbohydrates in subterranean tissue was linearly related to levels in shoots of *V. americana,* although the predictive capability of the relationship was poor

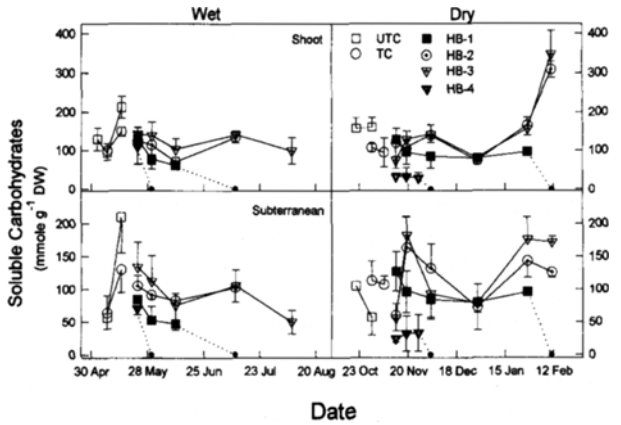

Fig. 9. Soluble carbohydrate levels measured in shoot and subterranean (roots + stolon) tissues of *Vallisneria americana* transplants sampled during wet and dry seasons. Measurements represent sucrose equivalents. Dotted line leading to small, filled circles indicates the plants were dead at the time of sampling. UTC = untransplanted, wild control; TC = transplanted control. Transplants were moved to experimental sites on the day the last UTC, TC samples were collected.

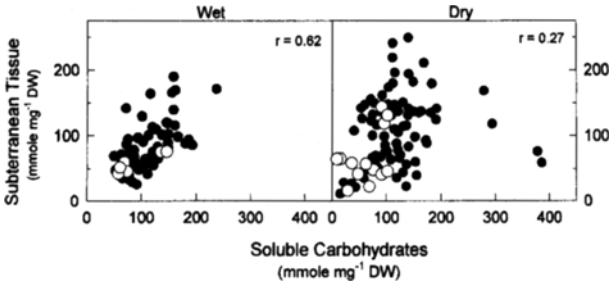

Fig. 10. Relationship between levels of soluble carbohydrates in shoots and in subterranean tissues of *Vallisneria americana* transplants sampled during wet and dry seasons. The unfilled circles represent measurements on plants at sites HB-1 and HB-4, 1–2 wk prior to death. The slopes of both regressions are significantly different from zero ($t_{wet} = 6.50$, $p < 0.001$; $t_{dry} = 2.87$, $p = 0.005$).

(Fig. 10; all treatments, $t_{wet} = 6.50$, $p < 0.001$, $r_{wet} = 0.62$; $t_{dry} = 2.87$, $p = 0.005$, $r_{dry} = 0.27$). Measurements made on HB-1 and HB-4 transplants 1–2 wk prior to death did not deviate from those obtained from healthy plants (open circles in Fig. 10).

Measured levels of insoluble carbohydrates were ca. 20% of levels of soluble carbohydrates. Insoluble carbohydrates did not provide evidence of physiological stress as early as did soluble carbohydrates (Fig. 11). For example, during the dry season, the soluble carbohydrate levels in both tissues declined within 1 wk after placement at site HB-4, but insoluble carbohydrate levels did not decline until 2–3 wk after placement.

There was some evidence that transplantation of *V. americana* was stressful to plant metabolism. Average levels of both soluble and insoluble carbohydrates were often higher in untransplanted, (wild) pre-experimental controls than in transplanted, pre-experimental controls (Figs. 9 and 11). However, when pooled by treatment (transplanted versus untransplanted controls), only levels of insoluble carbohydrates of shoot tissue collected during the wet season ($F = 7.38$, $p = 0.017$) and soluble carbohydrates of shoot tissue collected in the dry season ($F = 17.46$, $p < 0.001$) were statistically greater in the untransplanted controls.

Total C content of shoot and subterranean tissues of transplants maintained at the HB-2 and HB-3 sites varied only slightly over the course of the dry and wet season experiments (Fig. 12). Death of HB-4 plants during the wet season experiment occurred too rapidly to record any decrease in C content, although after 1 wk at the elevated salinities that characterize the site, the C content of HB-4 tissues ranked lowest of the four sites. During the dry season, the C content of HB-1 tissues prior to death did not demonstrate any perceptible pattern relative to HB-2 and HB-3. Tissue C content declined at site HB-4 after 1 wk in place until the last sampling of live plants. The loss of C was significant in subterranean tissue ($t = 4.75$, $p = 0.009$) though not in shoot tissue ($t = 2.29$, $p = 0.084$).

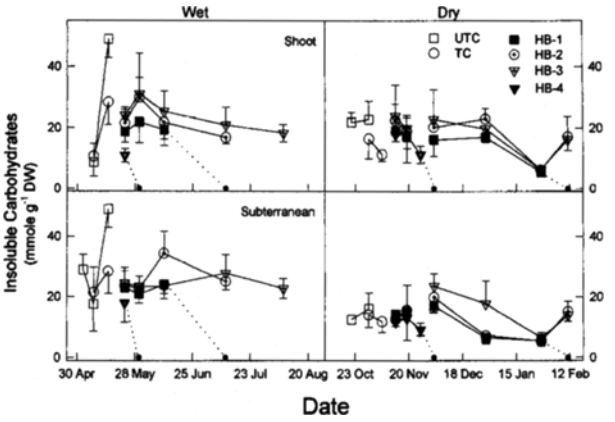

Fig. 11. Insoluble carbohydrate levels measured in shoot and subterranean (roots + stolon) tissues of *Vallisneria americana* transplants sampled during wet and dry seasons. Measurements represent sucrose equivalents. Dotted line leading to small, filled circles indicates the plants were dead at the time of sampling. UTC = untransplanted, wild control; TC = transplanted control. Transplants were moved to experimental sites on the day the last UTC, TC samples were collected.

Nitrogen-based Physiological Indices

During the wet season experiment, shoot glutamine synthetase (GS) activity provided an indication of stress prior to plant death at site HB-1; GS activity declined gradually before the death of the transplants. However, plants maintained at site HB-4 died before a physiological response could be

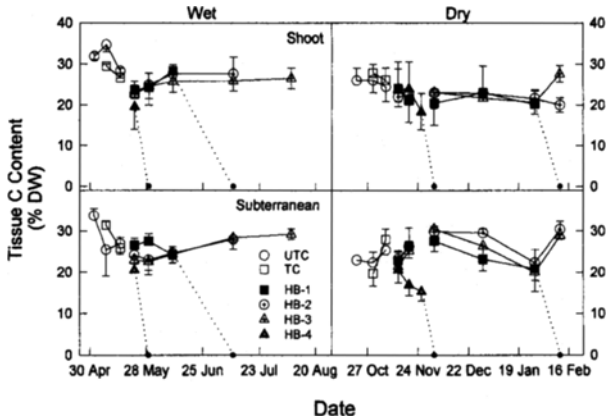

Fig. 12. Carbon content of shoots and subterranean (roots + stolons) tissue of *Vallisneria americana* transplants sampled during wet and dry seasons. Dotted line leading to small, filled circles indicates the plants were dead at the time of sampling. UTC = untransplanted, wild control; TC = transplanted control. Transplants were moved to experimental sites on the day the last UTC, TC samples were collected.

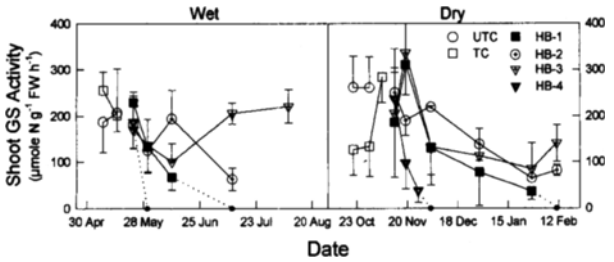

Fig. 13. Glutamine synthetase activity of shoots of *Vallisneria americana* transplants sampled during wet and dry seasons. Dotted line leading to small, filled circles indicates the plants were dead at the time of sampling. UTC = untransplanted, wild control; TC = transplanted control. Transplants were moved to experimental sites on the day the last UTC, TC samples were collected.

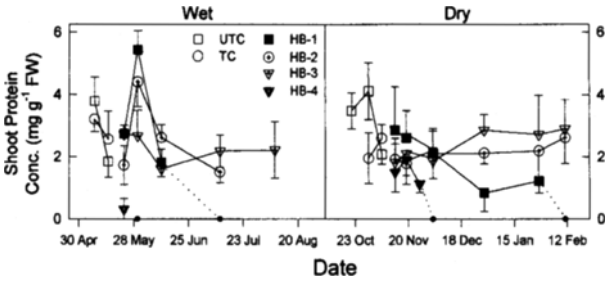

Fig. 14. Protein content of shoots of *Vallisneria americana* transplants sampled during wet and dry seasons. Dotted line leading to small, filled circles indicates the plants were dead at the time of sampling. UTC = untransplanted, wild control; TC = transplanted control. Transplants were moved to experimental sites on the day the last UTC, TC samples were collected.

measured (Fig. 13). During the dry season, declines in GS activity were evident at both HB-1 and HB-4, though the decline was much more rapid at the latter site. By the end of the second week of the dry season experiment, shoot GS activity at site HB-4 was significantly lower than at sites HB-1 and HB-3 ($F = 7.98$, $p = 0.002$).

Protein levels were similar in shoots collected from sites HB-2 and HB-3 (Fig. 14) during the wet season. Shoot protein levels declined, indicating physiological stress in transplants maintained at site HB-4 but not at site HB-1. Shoots maintained at site HB-4 during the wet season had significantly lower protein levels 1 wk post-placement than did shoots from the other sites ($F = 17.7$, $p < 0.001$). During the dry season, HB-4 shoot protein levels declined prior to transplant death; however, the difference between HB-4 and the less saline sites was not significant during the first 2 wk after placement ($F = 2.08$, $p = 0.14$). Protein levels in shoots maintained at site HB-1 declined prior to death.

The shoots at HB-1 prior to death had higher N levels than shoots from sites HB-2 or HB-3 during the wet season ($F = 36.9$, $p < 0.001$), though not during the dry season ($F = 1.26$, $p = 0.30$; Fig. 15). During the dry season the N content of shoots and subterranean tissue collected 3 wk after placement at site HB-4 (just prior to transplant death) had fallen 17% from levels measured 1 wk after placement at the site, and were 30% lower than levels of transplanted controls. The N content of shoots and subterranean tissue at sites HB-2 and HB-3 did not show a consistent pattern of change.

Discussion

Variations in freshwater input into the Caloosahatchee Estuary, including discharges from Lake Okeechobee, alter the salinity regime to which *V. americana* is exposed. While this suggests that salinity has major impacts on the biology of *V. americana*, we recognize that other factors co-vary with salinity and may also play a role in determining the distributional limits of SAV in the Caloosahatchee Estuary (Table 1). In our study, temperature did not control the physiological health and survival of the *V. americana* transplants in the Caloosahatchee Estuary; variation in temperature among the sites during each sampling date was minimal, and variation over time within each season was virtually identical at the four sites.

We conclude that the downstream limit to the distribution of *V. americana* (somewhere between sites HB-3 and HB-4) is determined by an upper salinity tolerance of > ca. 15‰. In mesocosm experiments, *V. americana* survived 15‰ for 6 wk, although no net growth was observed (Doering unpublished data). These tolerances are higher than

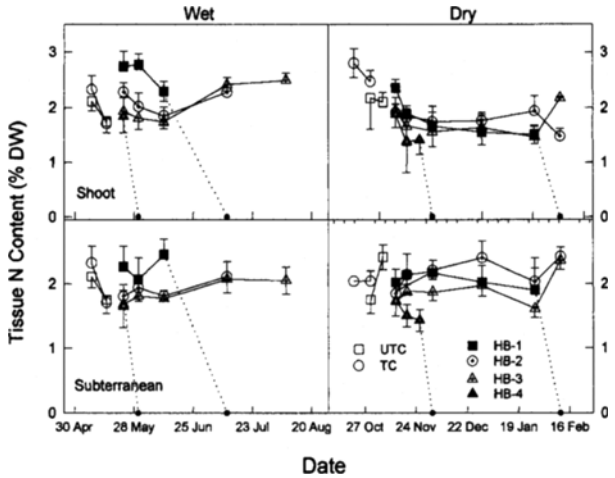

Fig. 15. Nitrogen content of shoots and subterranean (roots + stolons) tissue of *Vallisneria americana* transplants sampled during wet and dry seasons. Dotted line leading to small, filled circles indicates the plants were dead at the time of sampling.UTC = untransplanted, wild control; TC = transplanted control. Transplants were moved to experimental sites on the day the last UTC, TC samples were collected.

TABLE 1. Summary of the physical environment and the survival of *Vallisneria americana* at the four sites along the Caloosahatchee Estuary over the course of the wet (summer) and dry (winter) season experiments.

	Wet Season				Dry Season			
Site	Light[1]	Salinity[2]	Temp[3]	Survival[4]	Light	Salinity	Temp	Survival
HB-1	38%	0%	30	4–8	43%	0%	22	10–12
HB-2	20%	0%	29	>8**	13%	10%	22	>12
HB-3	25%	0%	30	>12	0%	50%	22	>12
HB-4	12%	33%*	30	<2	0%	80%	22	3–4

[1] % of observations where water column attenuation coefficient (k) > 3.5 m $^{-1}$.
[2] % of observations where salinity > 16‰.
[3] Average temperature during experiment.
[4] Weeks.
* Elevated salinities occurred at the outset of the experiment.
** Plants alive after 8 weeks at site; early loss of pots prevented collection at 12 weeks.

the highest published value (12‰; Twilly and Barko 1990). At site HB-4, light may be discounted as the downstream limiting factor because average water-column clarity increased in the downstream direction during the dry season. Although the average water-column attenuation coefficient did not vary significantly as a function of site during the wet season, the incidence of high turbidity events decreased in the downstream direction (Table 1). At site HB-4, salinity stresses during both wet and dry seasons caused rapid (1–4 wk) losses of physiological function and decreases in protein and carbohydrate contents in both aboveground and belowground tissues, leading to eventual death.

Photosynthetic rates of plants at site HB-4, however, were not altered prior to death. This is puzzling because any general metabolic disruption such as that engendered by hypersaline stress would be expected to disrupt photosynthetic metabolism (e.g., Macler 1988) as well as that involved in N assimilation (the GS enzyme system). However, no general consensus exists regarding the effects of salinity on photosynthesis by aquatic macrophytes. Although Kerr and Strother (1985) reported a decrease in the photosynthetic rate of the seagrass *Zostera muelleri* at elevated salinities, the change does not appear significant. Dawes et al. (1987) demonstrated a salinity effect on the photosynthetic rate of *Halophila engelmanii,* but no apparent effect was detected for *Ruppia maritima* (Lazar and Dawes 1991). Shoots of *V. americana* may be adapted to maintain photosynthetic output as long as possible under hypersaline stress.

Since the salinity regime measured at site HB-4 was not static during the periods preceding the death of *V. americana,* the lethal salinity can only be loosely defined as > 15‰. Plant death occurred in 1–2 wk postplacement during the wet season experiment, when salinities were decreasing from 20 to 17‰. Death during the dry season experiment occurred after 2 to 4 weeks of exposure to salinities that were increasing from 15‰ to 22‰. In addition to differences between the two experiments in the salinity regimes, developmental differences may be responsible for different salinity tolerances; transplants were still small at the outset of the wet season (blade length ca. 4–5 cm), while the dry season transplants were collected at the end of the growing season when they were larger (ca. 8–10 cm) and possibly more resistant. Doering et al. (unpublished data) examined several biological indices and reported nonlinear responses to varying salinity during the dry season, in contrast to the linearity observed in the wet season. Twilly and Barko (1990) suggested that salinity tolerance by freshwater angiosperms may be enhanced by a period of gradual acclimation to higher salinities, during which an amino acid-based osmoregulatory mechanism functions.

Barko et al. (1982) noted that the tolerance of *V. americana* to elevated temperatures increased with increasing irradiance. Light may also moderate hypersaline stress by providing additional energy to maintain an acceptable osmotic potential. Data presented here support this hypothesis; plants maintained at sites HB-2 and HB-3 during the dry season were virtually identical physiologically and similar in tissue C, carbohydrate, protein, and N content. Yet, the water-column salinities during the dry season at HB-3 exceeded those at HB-2 by 2.3–4.4‰ (see Table 1). We expect such an elevation in salinity to be biologically significant since HB-2 salinities were already high (avg = 12‰ for the experiment). Site HB-3, however, by virtue of its downstream location, experienced on average 33% greater photon flux densities than HB-2 during the dry season (calculated from average water-column extinction coefficients). Since energy and fixed C are required for osmotolerance, the in situ population of *V. americana* at HB-3 may owe its existence to increased water-column clarity (i.e., increased irradiance).

The mechanism(s) by which *V. americana* tolerates suboptimal salinities was not determined in these experiments. However, we speculate that N metabolism was altered qualitatively to provide some measure of tolerance. *Ruppia cirrhosa* (brackish water angiosperm), *Zostera capensis, Halodule wrightii, Thalassia testudinum,* and *Halophila engelmanii* (all seagrasses) employ proline as an osmolyte under hypersaline conditions (Pulich 1986; Adams and Bate 1994).

It is clear that the salinity tolerances of *V. americana* need to be revised. *V. americana* is commonly described as a freshwater angiosperm, with a low salinity tolerance (e.g., 7–8‰ limit to growth;

Bourn 1932; Day et al. 1989). Although much research has focused on northern and midwestern populations, the elevated salinity tolerance that we observed was probably not the result of ecotypic differentiation of a southern population; in a study of another *V. americana* population from southern Florida, Haller et al. (1974) reported that immersion in salinities 10‰ or greater resulted in plant death during a 4-wk experimental period. In this study *V. americana* survived for up to 12 wk at salinities that ranged from 12‰ to 20‰ (HB-3 site) and survived 4–6 weeks when salinities exceeded 15‰, even following an abrupt salinity transition. Whether *V. americana* could survive and reproduce after longer exposures to elevated salinity is not known. However, this field study, and laboratory-based mesocosm experiments by Doering (unpublished data), demonstrate the remarkable halotolerance of which *V. americana* is capable.

The furthest upstream site in the Caloosahatchee Estuary (HB-1) is, theoretically, more hospitable to *V. americana* than the donor site (HB-2) because of lower salinity. There was no resident population at HB-1, although *V. americana* was present adjacent to HB-1, in shallower water. This observation, coupled with the physiological and field environmental data, implicates reduced light availability in the death of the experimental transplants at HB-1, and highlights the role that light plays in determining the distribution of *V. americana* (Carter and Rybicki 1990; Carter et al. 1996). Reduced levels of soluble and insoluble carbohydrates at this site in both wet and dry seasons suggest that light was limiting. N assimilation also requires energy; the gradual decline of GS activity in transplants from site HB-1 over the course of both experiments likewise suggests light limitation.

Reduced irradiance may have been one contributor to the death of the transplants (and the general absence of a *V. americana* population) at site HB-1. The widespread elimination of *V. americana* populations in the Potomac River during the 1930s has been attributed to storm-induced sedimentation (Rybicki and Carter 1986); weather in general has been recognized as having important effects on light availability (Carter et al. 1994). In the present study, an episode of sediment deposition was recorded at HB-1 during the dry season. Partial tissue burial, reducing photosynthetic potential, may also have caused tissue anoxia, a significant stressor in its own right (Kozlowski 1984). A reduction in photosynthetic output, coupled with tissue anoxia, represent additional stresses that the already C-limited plants could not tolerate.

The demise of shoot and subterranean tissues may have resulted from the intracellular accumulation of NH_4^+. Lin and Kao (1996) showed that the capacity of rice roots to assimilate inorganic N via the GS enzyme system decreased under hypersaline conditions, leading to an accumulation of NH_4^+ in roots. Pulich (1986) reported a similar increase in shoot NH_4^+ in a seagrass maintained under hypersaline conditions. Metabolism and growth suffer when NH_4^+ is a major source of inorganic N, and death occurs when intracellular concentrations increase beyond low levels (Mehrer and Mohr 1989). Roots are clearly more at risk than are shoots, since the former are embedded in a sediment matrix that is generally high in NH_4^+ (e.g., Kenworthy et al. 1982).

Shoot GS activity provided a more precise indication of stress than did indices of C metabolism. Shoot GS activity showed clear evidence of a progressive decline in physiological function at sites HB-1 and HB-4 over both wet and dry seasons. Shoot protein also indicated extreme salinity stress at HB-4. In fact, shoot protein content may represent a rapid and efficacious measure of stress in *V. americana* populations. This putative stress response should, however, be investigated to determine its generality. Some stresses (e.g., light limitation of C metabolism) might not manifest themselves as reductions in protein content. Interestingly, at the two sites at which transplant death occurred, there was no metabolic "smoking gun" observed in this study; both C and N metabolism showed similar stress responses. Linkage between the C and N metabolic systems is well established (Turpin 1991; Turpin et al. 1991).

Overall, we found *V. americana* to be physiologically robust under a wide range of field conditions. The physiological measurements provided indications of stress but could not alone distinguish between light (HB-1) and salinity stresses (HB-4). However, deleterious metabolic effects translate into reductions in growth and survival, and hence help explain the absence of *V. americana* populations at the upstream and far downstream sites. Further understanding of the natural controls of SAV distribution must involve the study of the temporal responses to environmental fluctuations (light, salinity, etc.). For example, superimposed on the overall estuarine salinity gradient are periodic salinity oscillations. Montague and Ley (1993) reported that mean benthic plant biomass in northeast Florida Bay was correlated with the standard deviation in salinity rather than with average salinity. As the continuous record of salinity (Fig. 4) showed, significant changes in salinity can occur over time scales of days. The biological significance of short-term variations in salinity or other environmental parameters remains to be elucidated.

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