

## Biophysical and morphological leaf adaptations to drought and salinity in salt marsh grasses

Brian R. Maricle<sup>a,\*</sup>, Douglas R. Cobos<sup>b</sup>, Colin S. Campbell<sup>b</sup>

<sup>a</sup> School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA

<sup>b</sup> Decagon Devices, Inc., 950 NE Nelson Ct. Pullman, WA 99162, USA

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### Abstract

Leaf energy budgets were constructed for 13 species of estuarine  $C_4$  grasses (Poaceae) to elucidate the biophysical effects of drought and salinity on the interception and dissipation of solar energy. *Spartina alterniflora*, *S. anglica*, *S. argentinensis*, *S. bakeri*, *S. cynosuroides*, *S. densiflora*, *S. foliosa*, *S. foliosa* × *S. alterniflora* hybrids, *S. gracilis*, *S. patens*, *S. pectinata*, *S. spartinae*, and *Distichlis spicata* plants were grown under controlled soil water potential gradients in a greenhouse. Species were grouped into four major ecological functional types, based on elevational zonation ranges: low marsh species, middle marsh species, high marsh species, and freshwater species. Different functional types are adapted to different environmental conditions, and responded differently to reduced water potentials. Latent heat flux decreased similarly across species in response to decreasing water potential. Latent heat loss was found to decrease by as much as 65% under decreasing water potential, leading to an increase in leaf temperature of up to 4 °C. Consequently, radiative and sensible heat losses increased under decreasing water potential. Sensible heat flux increased as much as 336% under decreasing water potential. Latent heat loss appeared to be an important mode of temperature regulation in all species, and sensible heat loss appeared to be more important in high marsh species compared to low marsh species. High marsh species are characterized by narrower leaves than middle and low marsh species, leading to a smaller boundary layer, and providing higher conductance to sensible heat loss. This may be an adaptation for high marsh species to regulate leaf temperature without access to large amounts of water for transpirational cooling. Stomatal conductance decreased with decreasing water potential across species: leaf conductances to water vapor and  $CO_2$  decreased as much as 69% under decreasing water potential. Additionally, oxidative stress appeared to increase in these plants during times of drought or salinity stress. Ascorbate peroxidase activities increased with decreasing soil water potential, indicating increased cellular reactive oxygen species. High marsh species had higher ascorbate peroxidase activities compared to low marsh species, indicating higher tolerance to drought- or salinity-induced stresses. It was concluded that different species of marsh grasses are adapted for growth in different zones of salt marshes. Adaptations include biophysical, biochemical, and morphological traits that optimize heat exchange with the environment.

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**Keywords:** Adaptation; Ascorbate peroxidase; *Distichlis spicata*; Energy balance; Salt stress; *Spartina*; Water stress

**Abbreviations:**  $A$ ,  $CO_2$  assimilation rates; APX, ascorbate peroxidase;  $C_a$ , ambient concentration of  $CO_2$ ;  $C_i$ , intercellular concentration of  $CO_2$ ;  $c_p$ , specific heat of air;  $d$ , characteristic dimension of leaf;  $D$ , vapor pressure deficit;  $e_s$ , saturation vapor pressure;  $E$ , transpiration;  $F$ , view factor (proportion of surroundings occupied by an energy source);  $g_{CO_2}$ , leaf conductance to  $CO_2$ ;  $g_{Ha}$ , leaf boundary layer conductance to heat;  $g_{Hr}$ ,  $g_{Ha} + g_r$ ;  $g_r$ , radiative conductance;  $g_s$ , stomatal conductance;  $g_v$ , total leaf conductance to water vapor;  $g_{va}$ , boundary layer conductance to water vapor;  $g_{vs}$ , stomatal conductance to water vapor;  $H$ , sensible heat loss;  $L_a$ , longwave radiation emitted from air;  $L_g$ , longwave radiation emitted from ground;  $m$ , optical air mass;  $p_a$ , atmospheric air pressure; PPF, photosynthetic photon flux density; PSI, photosystem I;  $R$ , ideal gas constant;  $R_{abs}$ , total absorbed radiant energy;  $R_{net}$ , net radiation;  $s$ , slope of the saturation mole fraction function;  $S_b$ , direct beam of shortwave solar energy;  $S_d$ , diffuse shortwave radiation;  $S_{po}$ , solar constant;  $S_r$ , reflected shortwave radiation;  $S_t$ , total shortwave radiation;  $T_a$ , air temperature;  $T_L$ , leaf temperature;  $T_w$ , wet bulb temperature;  $u$ , wind speed;  $\alpha_s$ , shortwave absorptivity;  $\alpha_L$ , longwave absorptivity;  $\gamma^*$ , psychrometric constant;  $\epsilon$ , longwave emissivity;  $\theta$ , solar zenith angle;  $\lambda$ , latent heat of vaporization of water;  $\lambda E$ , latent heat loss;  $\rho_s$ , shortwave surface reflectance (albedo);  $\sigma$ , Stefan-Boltzmann constant;  $\tau$ , atmospheric transmittance;  $\Psi$ , water potential;  $\Psi_{soil}$ , water potential of soil;  $\Psi_{solution}$ , water potential of nutrient solution; ‰, salinity in parts per thousand

\* Corresponding author. Present address: Department of Biological Sciences, Fort Hays State University, 600 Park St., Hays, KS 67601-4099, USA.  
Tel.: +1 785 628 5822; fax: +1 785 628 4153.

E-mail address: [brmaricle@fhsu.edu](mailto:brmaricle@fhsu.edu) (B.R. Maricle).

## 1. Introduction

The salinity of sediment porewater is an important environmental factor influencing plant growth and species composition in estuarine salt marshes. Salt ion toxicity has numerous deleterious effects on plants such as denaturing cytosolic enzymes (Munns, 2002) and facilitating the formation of reactive oxygen species that can damage membranes and proteins (Zhu, 2001). Like drought stress, increasing soil salinity involves a decrease in soil water potential ( $\Psi$ ) (Hasegawa et al., 2000). Stomata are sensitive to changes in soil water potential, so stomatal closure usually accompanies drought and salt stress (Brugnoli and Lauteri, 1991). A decrease in stomatal conductance reduces incoming  $\text{CO}_2$  and thus can reduce photosynthetic rates.

Even in halophytic salt marsh species,  $\text{CO}_2$  fixation rates are sensitive to increasing salinity levels (reviewed by Drake, 1989). While  $\text{CO}_2$  fixation rates typically decrease in times of salt stress, chlorophyll fluorescence analysis has revealed that light-harvesting processes are not affected by high salinity in some marsh grass species (Nieva et al., 1999). This results in a surplus of light energy that is not used in photosynthesis. Without safe dissipation, this excess light energy can result in over-reduction of electron carriers and the subsequent formation of reactive oxygen species (Demmig-Adams and Adams, 1992). As a result, plant antioxidant systems can also play an important role in times of salinity stress (Zhu, 2001). Reduced photosynthetic production resulting from increased salinity can therefore result from toxic salt ion effects, decreased  $\text{CO}_2$  fixation from closure of stomata, or oxidative stress from reactive oxygen species.

Intertidal estuarine zones are inundated with brackish water twice daily (Pennings and Bertness, 2001), so fluctuating salt levels and fluctuating water potentials are important physical factors influencing plant productivity in estuaries. However, the effects of soil salinity and drought conditions on the biophysical energy budgets of salt marsh grasses remain unknown. Although many earlier studies have investigated environmental effects on energy budgets of flood-tolerant plants like rice (Homma et al., 1999; Campbell et al., 2001; Oue, 2001) or other wetland communities (e.g., Bracho and Jose, 1990; Lafleur, 1990; Goodin et al., 1996; Souch et al., 1998; Jacobs et al., 2002), much less work has been done with halophytes. Previous work has examined the importance of latent heat flux (Teal and Kanwisher, 1970) and effects of water level on marsh energy budgets (Heilman et al., 2000), but no previous studies have measured leaf energy budgets of salt marsh plants over a controlled water potential gradient.

In the present study, we investigated the biophysical effects of decreasing water potential on energy interception and dissipation processes in salt marsh grasses. Leaf energy budgets were calculated for the estuarine  $\text{C}_4$  grasses *Spartina alterniflora* Loisel., *S. anglica* C. E. Hubbard, *S. argentinensis* (Trin.) Parodi, *S. bakeri* Merr., *S. cynosuroides* (L.) Roth, *S. densiflora* Brongn., *S. foliosa* Trin., *S. foliosa*  $\times$  *S. alterniflora* hybrids, *S. gracilis* Trin., *S. patens* (Aiton) Muhl., *S. pectinata* Bosc ex Link, *S. spartinae* (Trin.) Merr. ex A.S. Hitchc, and *Distichlis spicata* (L.) Greene under controlled drought and salinity conditions in

a greenhouse. Incoming solar energy was measured and outgoing radiative, latent, and sensible heat losses were calculated to examine the effects of sediment water status on the biophysical absorption and dissipation of radiant energy. Additionally, leaf conductances to  $\text{CO}_2$  were calculated to assess physiological responses of plants to decreasing soil water potential. We hypothesized that decreased stomatal conductance resulting from drought or salinity stress would decrease latent heat flux and shift that portion of the heat load on radiative and sensible heat losses. Sensible heat loss may become more important for leaf temperature regulation in plants adapted for growth in high marsh areas with higher salinity stress and less water available, and this may cause morphological changes to adapt to these conditions.

Levels of oxidative stress were also investigated in plants with assays of leaf ascorbate peroxidase (APX). Previous studies have found  $\text{CO}_2$  fixation rates in marsh plants to be very sensitive to increasing salinity (reviewed by Drake, 1989), but the biophysical light-harvesting processes are normally not affected by increasing salinity (e.g., Nieva et al., 1999). When  $\text{CO}_2$  fixation rates are limited relative to light harvesting rates (e.g., under water stress), electron carriers can become over-reduced, and  $\text{O}_2$  can be reduced to the highly reactive  $\text{O}_2^-$  at PSI. Superoxide dismutase oxidizes  $\text{O}_2^-$  and forms  $\text{H}_2\text{O}_2$ , which APX then reduces to  $\text{H}_2\text{O}$ . This forms the “water–water cycle” in chloroplasts (Asada, 1999) and can become important under conditions where  $\text{CO}_2$  fixation rates become limited relative to light harvesting rates (Demmig-Adams and Adams, 1992). A combination of decreased stomatal conductance and increased oxidative stress were expected to decrease photosynthetic  $\text{CO}_2$  conductances and help account for decreased productivity under salt or drought stress.

The group of estuarine grasses tested in this study represents a range of ecological functional types including low intertidal marsh species (*S. alterniflora* and *S. anglica*), middle marsh species (*S. cynosuroides*, *S. densiflora*, *S. foliosa*, and *S. foliosa*  $\times$  *S. alterniflora* hybrids), high intertidal marsh species (*S. argentinensis*, *S. bakeri*, *S. patens*, *S. spartinae*, and *D. spicata*), and inland freshwater species with lower salt tolerance (*S. gracilis* and *S. pectinata*). These estuarine zones impose different physical conditions on plant life, so an elevational gradient across an intertidal salt marsh contains an assortment of plant species that encounter different salinity and waterlogging regimes in their natural environments. Low intertidal marsh species are regularly inundated with tides; plants in these areas are adapted to waterlogged sediments with moderate to high salinity (Bertness and Ellison, 1987). In contrast, high intertidal marsh species experience infrequent tidal fluxes. Evaporative stress is often high in these areas, leading to drier soils often containing higher salinity levels compared to low marsh regions (Pennings and Callaway, 1992). Thus high marsh plants are adapted to high salt levels but not chronic waterlogging.

This study was conducted to examine the biophysical responses of salt marsh plants to increasing drought and salt stress. The results of this study are expected to be of broad interest because many plants are confronted with drought or salt stress (low  $\Psi$ ) during part of their life. Historically, over 25% of

Table 1

The plant species included in the study. Shown is the approximate ecological functional type of each species, after Mobblerley (1956) and other sources as stated, and where each was collected

Species	Ecological functional type	Collected from
<i>Spartina alterniflora</i>	Low marsh <sup>a,b,c</sup>	Willapa Bay, Washington
<i>Spartina anglica</i>	Low marsh <sup>d,b</sup>	Puget Sound, Washington
<i>Spartina argentinensis</i>	High marsh	Argentina
<i>Spartina bakeri</i>	High marsh	Sapelo Island, Georgia
<i>Spartina cynosuroides</i>	Middle marsh <sup>e,f</sup>	Sapelo Island, Georgia
<i>Spartina densiflora</i>	Middle marsh <sup>g</sup>	Odeil Salt marshes, SW Spain
<i>Spartina foliosa</i>	Middle marsh <sup>h,i</sup>	San Francisco Bay, California
<i>S. alterniflora</i> x <i>S. foliosa</i> F1 hybrid	Middle marsh <sup>i,j</sup>	San Francisco Bay, California
<i>Spartina gracilis</i>	Inland freshwater	Grant County, Washington
<i>Spartina patens</i>	High marsh <sup>c,k</sup>	Gulf Coast, NW Florida
<i>Spartina pectinata</i>	Inland freshwater <sup>k</sup>	Butler County, Nebraska
<i>Spartina spartinae</i>	High marsh	Galveston Bay, Texas
<i>Distichlis spicata</i>	High marsh <sup>h,k</sup>	Puget Sound, Washington

<sup>a</sup> McKee and Patrick, 1988.

<sup>b</sup> Sayce and Mumford, 1990.

<sup>c</sup> Bertness, 1991.

<sup>d</sup> Frenkel, 1987.

<sup>e</sup> Odum and Fanning, 1973.

<sup>f</sup> Higinbotham et al., 2004.

<sup>g</sup> Castillo et al., 2000.

<sup>h</sup> Zedler et al., 1999.

<sup>i</sup> Ayres et al., 2004.

<sup>j</sup> Maricle, personal observation.

<sup>k</sup> Nova Scotia Museum of Natural History, 1996.

U.S. soils are prone to drought conditions and 40.8% of insurance claims for crop loss in the U.S. result from drought (Boyer, 1982). Additionally, almost half the irrigated land and 20% of the world's cultivated lands are subjected to some degree of salinity (Zhu, 2001). Adaptations of plants that naturally tolerate low water potentials are clearly important when considering primary productivity in many estuarine and agricultural systems.

## 2. Materials and methods

### 2.1. Plant material and growing conditions

Experimental plants were collected from the field sites listed in Table 1 and were subsequently maintained and tested under greenhouse conditions. Field plants were broken apart and tillers were potted individually in a 50/50 (v/v) sand/potting

soil mixture. All plants were watered sparingly twice weekly with modified Hoagland nutrient solution (Epstein, 1972). Greenhouse conditions consisted of natural lighting (average PPFD was 1280  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and average radiant energy was 480  $\text{W m}^{-2}$  at midday) with 26 °C daytime temperatures and 18 °C at night.

Plants were randomized between treatments (drained vs. flooded soil conditions; 0 to 40‰ salt) and allowed 30 days to acclimate to growing conditions before initiating the experiment. Twelve pots were placed into each tub in an unbalanced block design; at least five tubs were used for each treatment. Flooded treatment plants were submerged to a level 2 cm above the soil surface (about 12 L) and the water was completely replaced weekly. During the acclimation period, salinity levels were increased incrementally (10‰ per week) until flooded treatments included 0, 10, 20, 30, and 40‰ salt (Instant Ocean salts; Aquarium Systems, Mentor, OH). Drained treatment plants represented droughted conditions. These plants were watered sparingly twice a week with water containing 0 or 10‰ salt; the surface soil in drained treatment plants became slightly dry between waterings. Once proper salinity levels were reached, plants grew at least 30 days in their respective treatment before testing. The freshwater species *S. gracilis* and *S. pectinata* were quite sensitive to salt, and most of these plants died in treatments with at least 20‰ salt. This led to small sample sizes ( $n=1$ ) for the “freshwater” functional type in the 40‰ treatment, and  $n=3-6$  in all other salinity levels >0‰ in the “freshwater” functional type. The statistical model was therefore not able to compare 40‰ salinity freshwater plants with other species or treatments. The other three species groupings had substantially larger sample sizes across treatments ( $n=9-91$ ).

### 2.2. Water potential measurements

A vapor pressure osmometer (model 5500; Wescor, Inc., Logan, UT) was used to measure osmolality ( $\text{mmol kg}^{-1}$ ) of nutrient solutions (Ball and Oosterhuis, 2005). 10  $\mu\text{L}$  samples from six replicate mixings were measured for each salinity level prior to addition to plants, and  $\Psi$  was calculated using the Van't Hoff relation:

$$\Psi(\text{MPa}) = -RT(\text{mol kg}^{-1}) \quad (1)$$

where  $R$  is the ideal gas constant ( $0.00831 \text{ MPa kg mol}^{-1} \text{ K}^{-1}$ ) and  $T$  is temperature (K) (Nobel, 1983). Solution  $\Psi$  was very consistent between mixings (Table 2).

Table 2

Solution and soil water potentials ( $\Psi$ ) of all waterlogging and salinity treatments used in the study. Shown is the mean  $\pm$  S.D. ( $n$ ) for each treatment. Treatments are identified by salinity (‰) and “flooded” or “drained” soil conditions

Nutrient solution salinity (‰)	$\Psi_{\text{solution}}$ (MPa)	Treatment	$\Psi_{\text{soil}}$ (MPa)
0‰	$-0.034 \pm 0.12$ (6)	0‰ flooded	$-0.195 \pm 0.081$ (21)
10‰	$-0.87 \pm 0.07$ (6)	0‰ drained	$-0.69 \pm 0.28$ (26)
20‰	$-1.59 \pm 0.04$ (6)	10‰ flooded	$-0.98 \pm 0.06$ (12)
30‰	$-2.38 \pm 0.04$ (6)	20‰ flooded	$-1.66 \pm 0.07$ (12)
40‰	$-3.05 \pm 0.10$ (6)	30‰ flooded	$-2.34 \pm 0.06$ (12)
		40‰ flooded	$-3.11 \pm 0.20$ (9)
		10‰ drained	$-5.03 \pm 1.87$ (8)

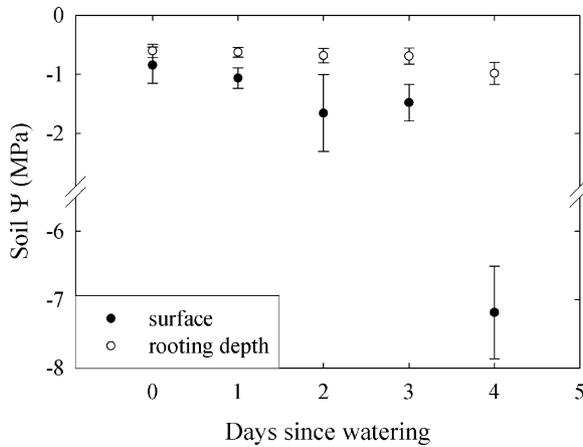


Fig. 1. The water potential ( $\Psi$ ) of soils in 0% drained treatments at surface (black circles) and rooting depth (open circles). Bars indicate  $\pm$  S.E. ( $n=4-6$ ).

Soil water potentials were measured for plants in all treatments using a WP4 Dew Point Potentiometer (Decagon Devices, Inc., Pullman, WA). Soil  $\Psi$  was measured at the surface and at rooting depths (4–5 cm) in 4–6 pots from each of two tubs per treatment following two separate watering events. Soil  $\Psi$  was very consistent between pots for flooded treatments (Table 2). Drained treatment plants were slightly more variable between individuals. Surface  $\Psi$  was quite variable between waterings in drained treatment plants, but  $\Psi$  at rooting depth remained nearly constant (Fig. 1).

### 2.3. Leaf energy budget calculations

Leaf energy budgets were constructed for three populations of greenhouse plants. Measurements were made on each plant during mid-day hours on clear days on 8 April to 16 April 2004, 23 May to 4 June 2005, and 24 August to 13 September 2005. A standard sling psychrometer (model #1328; Taylor precision products; Oak Brook, IL) was used to measure dry bulb ( $T_a$ ) and wet bulb temperatures ( $T_w$ ), and temperatures of the greenhouse walls and floor were measured with an infrared thermometer (Raytek Raynger ST; Total Temperature Instrumentation, Inc.; Williston, VT). Wind speed ( $u$ ) was measured with a Traceable® hot wire anemometer (Control Company; Friendswood, TX). Stomatal conductance ( $g_s$ ) was measured with an LI-1600 steady state porometer (LI-COR Inc., Lincoln, NE) and leaf temperature ( $T_L$ ) was measured for each plant with a fine-wire thermocouple inside the porometer leaf chamber. Total incoming shortwave radiant energy ( $S_t$ ) was measured with an LI-200 cosine-corrected pyranometer (LI-COR Inc., Lincoln, NE). Diffuse shortwave energy ( $S_d$ ) was calculated after Liu and Jordan (1960) as:

$$S_d(\text{W m}^{-2}) = 0.3(1 - \tau^m)S_{po}\cos\theta \quad (2)$$

where  $\tau$  is atmospheric transmittance (taken to be 0.7 on a clear day; Campbell and Norman, 1998),  $\theta$  is the solar zenith angle,  $S_{po}$  is the solar constant ( $1360 \text{ W m}^{-2}$ ), and  $m$  is the optical air mass, calculated after Campbell and Norman (1998) as:

$$m(\text{unitless}) = \frac{p_a}{(101.3 \text{ kPa})(\cos\theta)} \quad (3)$$

where  $p_a$  is atmospheric pressure (in kPa). Reflected shortwave radiation ( $S_r$ ) was calculated as:

$$S_r(\text{W m}^{-2}) = \rho_s(S_t) \quad (4)$$

where  $\rho_s$  is the shortwave surface reflectance (albedo), measured to be 0.166 in the greenhouse.

The direct beam of solar energy ( $S_b$ ) was calculated after Campbell and Norman (1998) as:

$$S_b(\text{W m}^{-2}) = S_t - S_d \quad (5)$$

Total absorbed radiant energy ( $R_{abs}$ ) was then calculated, modified from Campbell and Norman (1998) as:

$$R_{abs}(\text{W m}^{-2}) = \alpha_s(F_b S_b + F_d S_d + F_r S_r) + \alpha_L(F_a L_a + F_g L_g) \quad (6)$$

where  $\alpha_s$  and  $\alpha_L$  are absorptivities of plant leaves in the shortwave and longwave ranges;  $\alpha_s$  was estimated from pyranometer measurements of incident, transmitted, and reflected radiation from plant leaves, and  $\alpha_L$  was taken to be 0.97 (Campbell and Norman, 1998). The “ $F$ ” terms represent the appropriate view factor for each component:  $F_b$  is 0.5,  $F_d$  is 1.0, and  $F_r$ ,  $F_a$ , and  $F_g$  are each 0.5.  $L_a$  and  $L_g$  are longwave radiation emitted from the atmosphere and ground, calculated as:

$$L(\text{W m}^{-2}) = \varepsilon\sigma T^4 \quad (7)$$

where  $\varepsilon$  is the longwave emissivity of the object (taken to be 0.90 for concrete and 0.93 for glass; Campbell and Norman, 1998),  $\sigma$  is the Stefan-Boltzmann constant ( $5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$ ), and  $T$  is the temperature of the object (K). Net radiation was then found by subtracting the outgoing radiative (longwave) radiation from each plant:

$$R_{net}(\text{W m}^{-2}) = R_{abs} - \varepsilon_L\sigma T_L^4 \quad (8)$$

where  $\varepsilon_L$  is the emissivity of the leaf (taken to be 0.97; Campbell and Norman, 1998), and  $T_L$  is leaf temperature (K). The complete energy budget was then found by subtracting sensible and latent heat lost:

$$R_{net} - H - \lambda E = 0 (\text{W m}^{-2}) \quad (9)$$

with an experimental error rate of 2.91%. In the above equation,  $H$  is sensible heat flux and  $\lambda E$  is latent heat loss (Campbell and Norman, 1998). Sensible heat flux was calculated as:

$$H(\text{W m}^{-2}) = c_p g_{Ha}(T_L - T_a) \quad (10)$$

where  $c_p$  is the specific heat of air ( $29.3 \text{ J mol}^{-1} \text{ }^\circ\text{C}^{-1}$ ) and  $g_{Ha}$  is the leaf boundary layer conductance to heat, calculated as:

$$g_{Ha}(\text{mol m}^{-2} \text{ s}^{-1}) = (0.135)\sqrt{\frac{u}{d}} \quad (11)$$

where  $u$  is the wind speed ( $\text{m s}^{-1}$ ) and  $d$  is the characteristic dimension of the leaf ( $m$ ), found by multiplying 0.72 by the maximum width of the plant leaf (Campbell and Norman, 1998).

Latent heat flux ( $\lambda E$ ) was found using the Penman-Monteith equation (Monteith, 1965):

$$\lambda E_{\text{leaf}}(\text{W m}^{-2}) = \frac{s(R_{\text{abs}} - \varepsilon_L \sigma T_a^4) + \gamma^* \lambda g_v D / P_a}{s + \gamma^*} \quad (12)$$

where  $g_v$  is leaf conductance to water vapor,  $T_a$  is in K,  $D$  is the vapor pressure deficit (kPa),  $p_a$  is atmospheric pressure (kPa),  $s$  is the slope of the saturation mole fraction function, and  $\gamma^*$  is the psychrometric constant, calculated as:

$$s(^{\circ}\text{C}^{-1}) = \frac{(17.502)(265.5^{\circ}\text{C})(e_s)}{(p_a)(265.5^{\circ}\text{C} + T_a)^2} \quad (13)$$

$$\gamma^*(^{\circ}\text{C}^{-1}) = \frac{c_p/\lambda \times g_{\text{Hr}}}{g_v} \quad (14)$$

where  $\lambda$  is the latent heat of vaporization of water ( $44,000 \text{ J mol}^{-1}$ ),  $e_s$  is the saturation vapor pressure at the measured air temperature, and  $g_{\text{Hr}} = g_{\text{Ha}} + g_{\text{r}}$  (Campbell and Norman, 1998). Radiative conductance ( $g_{\text{r}}$ ) was calculated as:

$$g_{\text{r}}(\text{mol m}^{-2} \text{ s}^{-1}) = \frac{4\varepsilon_L \sigma T_a^3}{c_p} \quad (15)$$

where  $T_a$  is in K (Campbell and Norman, 1998). Leaf conductance to water vapor ( $g_v$ ) is the sum of boundary layer and stomatal resistances and was calculated as:

$$g_v(\text{mol m}^{-2} \text{ s}^{-1}) = \frac{1}{1/g_{\text{vs}} + 1/g_{\text{va}}} \quad (16)$$

where  $g_{\text{vs}}$  is stomatal conductance to water vapor measured with the LI-1600 porometer and  $g_{\text{va}}$  is the boundary layer conductance to water vapor, calculated after Campbell and Norman (1998):

$$g_{\text{va}}(\text{mol m}^{-2} \text{ s}^{-1}) = (0.147) \sqrt{\frac{u}{d}} \quad (17)$$

Leaf conductance to  $\text{CO}_2$  was calculated after Salisbury and Ross (1992):

$$g_{\text{CO}_2} = 0.625 g_v \quad (18)$$

$g_{\text{CO}_2}$  measures allow researchers to calculate  $\text{CO}_2$  assimilation rates ( $A$ ) with simple models, e.g.,  $A = g_{\text{CO}_2}(C_a - C_i)$ , where  $C_a$  and  $C_i$  are ambient and intercellular concentrations of  $\text{CO}_2$  (Salisbury and Ross, 1992). Since  $\text{CO}_2$  levels within the greenhouse were likely not equal to ambient, we present only  $g_{\text{CO}_2}$  and not  $A$ .

#### 2.4. Leaf ascorbate peroxidase assays

Following energy budget measurements, plant leaves were harvested and flash-frozen in liquid nitrogen. Assays of the antioxidant enzyme ascorbate peroxidase (APX) were later performed spectrophotometrically after Nakano and Asada (1981). Leaf samples stored at  $-80^{\circ}\text{C}$  were ground with a mortar and pestle in  $10 \text{ mL g}^{-1}$  cold extraction buffer, containing  $50 \text{ mM}$  Tris (pH 7.0) with  $5 \text{ mM}$   $\text{MgCl}_2$ ,  $2 \text{ mM}$  cysteine hydrochloride, and  $2\%$  w/v PVP-40. The resulting mixture was centrifuged at  $15,000 \times g$  for 5 min at  $4^{\circ}\text{C}$ . The supernatant was assayed

for APX activity at  $25^{\circ}\text{C}$ .  $10 \mu\text{L}$  aliquots of the extract supernatant were added to  $0.980 \text{ mL}$  of a reaction mixture containing  $50 \text{ mM}$   $\text{KH}_2\text{PO}_4$ ,  $0.5 \text{ mM}$  ascorbate, and  $0.1 \text{ mM}$  EDTA (pH 7.0). Background rates of ascorbate reduction were calculated in the presence of enzyme extract but in the absence of  $\text{H}_2\text{O}_2$ . The reaction was initiated with the addition of  $10 \mu\text{L}$   $10 \text{ mM}$   $\text{H}_2\text{O}_2$  (final concentration =  $0.1 \text{ mM}$ ). Enzyme activity was determined as a decrease in ascorbate, measured as a decrease in absorbance at  $290 \text{ nm}$ , using an extinction coefficient of  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$  (Nakano and Asada, 1981). APX activities were corrected for background rates of ascorbate reduction and standardized to g fresh leaf weight.

#### 2.5. Statistical analyses

Statistical analyses were performed using two-factor (species and treatment) analysis of variance (proc mixed; SAS version 8.0, 2001 SAS Institute Inc., Cary, NC;  $\alpha = 0.05$ ). In these models species were grouped by ecological functional type and blocked by the three populations and the 76 tubs involved in the study.

### 3. Results

#### 3.1. Effects of decreasing water potential

Increasing salinity and increasing soil dryness both decreased soil water potential ( $\Psi$ ) and both had similar effects on plant energy budgets in this study. The soil in drained treatment plants became slightly dry between waterings (Fig. 1), thus reducing the matric potential of the soil (Salisbury and Ross, 1992). Plants growing in the  $10\%$  salt drained treatment experienced the most severe water stress from a combination of the low matric potentials of dry soils and low osmotic potentials characteristic of saline soils (Table 2). In contrast to the drained treatments, the soil in flooded treatment plants remained saturated throughout the experiment. Therefore, the matric potential of these soils remained at zero. The stepwise increase in treatment salinity resulted in a corresponding decrease in soil osmotic potential (Table 2). Consequently, both drought and saline conditions result in decreased soil  $\Psi$ , with an additive effect in the  $10\%$  salt drained treatment.

Leaf shortwave absorptivities ( $\alpha_s$ ) ranged from 0.62 to 0.70 across species (data not shown). There were no differences in  $\alpha_s$  between treatments or species (ANOVA,  $p > 0.126$ ; data not shown). Absorbed radiation ( $R_{\text{abs}}$ ) did not differ between treatments (ANOVA,  $p = 0.924$ ) or species groupings (ANOVA,  $p = 0.103$ ). Mean  $R_{\text{abs}}$  ranged from  $572.4$  to  $662.4 \text{ W m}^{-2}$  (Fig. 2a); variations in  $R_{\text{abs}}$  reflect changing time-of-day conditions between individual measurements. Net radiation ( $R_{\text{net}}$ ) was found by subtracting leaf radiative energy from  $R_{\text{abs}}$  (Eq. (8)). Therefore, plants with higher leaf temperatures had a correspondingly lower  $R_{\text{net}}$ . Mean  $R_{\text{net}}$  ranged from  $99.2$  to  $208.9 \text{ W m}^{-2}$  across treatments and species groupings (Fig. 2b) and did not differ between species groupings (ANOVA,  $p = 0.786$ ). While variations in  $R_{\text{net}}$  reflect the random variations in  $R_{\text{abs}}$ , some differences between treatments were apparent. Plants under drained soil conditions and higher salinities tended

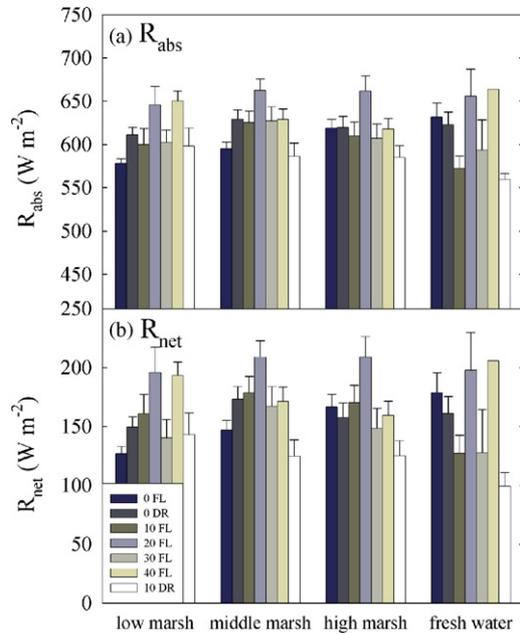


Fig. 2. The effect of soil  $\Psi$  on (a) total leaf absorbed shortwave radiation ( $R_{abs}$ ) and (b) leaf net radiation ( $R_{net}$ ). Bars indicate  $\pm$  S.E. ( $n=3-91$ ). Species are grouped by ecological functional type. Treatments are arranged by decreasing  $\Psi$ : the number for each treatment category (0 through 40) refers to treatment salinity (‰) and “DR” or “FL” refers to drained or flooded soil conditions.

to have the lowest  $R_{net}$  values.  $R_{net}$  significantly decreased with decreasing  $\Psi$  (ANOVA,  $p < 0.001$ ), resulting from increased leaf temperature ( $T_L$ ) in low  $\Psi$  treatments. Mean  $T_L$  ranged from 25.9 to 30.4 °C across treatments and species groupings (Fig. 3a).  $T_L$  did not differ between species groupings (ANOVA,  $p = 0.398$ )

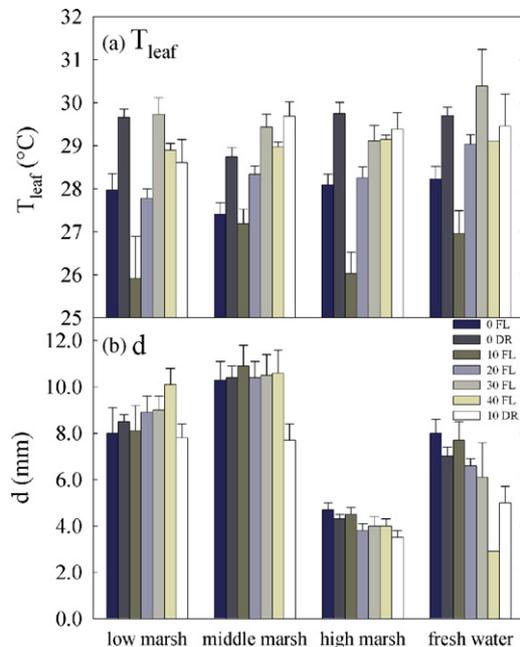


Fig. 3. The effect of soil  $\Psi$  on (a) leaf temperature ( $T_{leaf}$ ) and (b) leaf characteristic dimension ( $d$ ). Bars indicate  $\pm$  S.E. ( $n=3-91$ ). Species are grouped by ecological functional type; treatments are arranged by decreasing  $\Psi$  and are labeled as in Fig. 2.

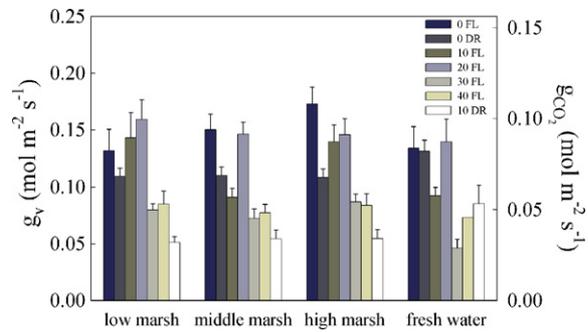


Fig. 4. The effect of decreasing soil  $\Psi$  on leaf conductance to water vapor ( $g_v$ ) and leaf  $CO_2$  conductance ( $g_{CO_2}$ ). Bars indicate  $\pm$  S.E. ( $n=3-91$ ). Species are grouped by ecological functional type; treatments are arranged by decreasing  $\Psi$  and are labeled as in Fig. 2.

but became significantly higher with decreasing  $\Psi$  (ANOVA,  $p < 0.001$ ), increasing by as much as 4 °C.

Mean leaf characteristic dimension ( $d$ ) ranged from 3.8 to 10.9 mm (Fig. 3b) and was significantly different between each of the four species groupings (high marsh < fresh water < low marsh < middle marsh; ANOVA,  $p < 0.001$ ). Although not significantly different between treatments (ANOVA,  $p = 0.265$ ), there appeared to be trends of  $d$  decreasing under decreasing  $\Psi$  (Fig. 3b), either as a gradual decline with decreasing  $\Psi$  (high marsh and fresh water) or as a threshold response (middle marsh).

Increasing soil dryness had a large effect on stomatal conductance ( $g_s$ ) and related parameters ( $g_v$ ,  $g_{CO_2}$ , and  $\lambda E$ ) in all species. Mean  $g_s$  measures ranged from 49  $mmol m^{-2} s^{-1}$  in low  $\Psi$  treatments up to 206  $mmol m^{-2} s^{-1}$  in high  $\Psi$  treatments (data not shown). There were no significant differences between species groupings (ANOVA,  $p = 0.403$ ), but large differences were observed between treatments in all species (ANOVA,  $p < 0.001$ ), suggesting similar effects across species. The decreased  $g_s$  resulting from decreasing  $\Psi$  led to consequent decreased leaf vapor conductance ( $g_v$ ) across species. Mean  $g_v$  ranged from 0.046 to 0.173  $mol m^{-2} s^{-1}$  across treatments and species groupings (Fig. 4). In a manner similar to  $g_s$ ,  $g_v$  did not differ between species groupings (ANOVA,  $p = 0.210$ ) but was strongly reduced at low  $\Psi$  (ANOVA,  $p < 0.001$ ).

Rates of latent heat loss ( $\lambda E$ ) were not significantly different between species groups (ANOVA,  $p = 0.671$ ) but were strongly affected by decreasing soil  $\Psi$  (ANOVA,  $p < 0.001$ ). Mean  $\lambda E$  ranged from 61.4  $W m^{-2}$  in water stressed plants up to 175.4  $W m^{-2}$  in moderate salinity plants (Fig. 5a).

Mean  $H$  ranged from 14.5 to 100.6  $W m^{-2}$  across treatments (Fig. 5b).  $H$  became significantly higher with decreasing soil  $\Psi$  (ANOVA,  $p = 0.001$ ). The high marsh species in this study showed significantly higher rates of sensible heat loss compared to middle and low marsh species (ANOVA,  $p = 0.030$ ).

The relation of sensible to latent heat losses is often expressed as the Bowen ratio ( $H/\lambda E$ ). Mean Bowen ratios ranged from 0.11 to 2.05 in the present study (Fig. 6). Bowen ratios did not differ between species groupings (ANOVA,  $p = 0.263$ ) but significantly increased with decreasing  $\Psi$  (ANOVA,  $p < 0.001$ ). A significant treatment/species grouping interaction ( $p = 0.022$ )

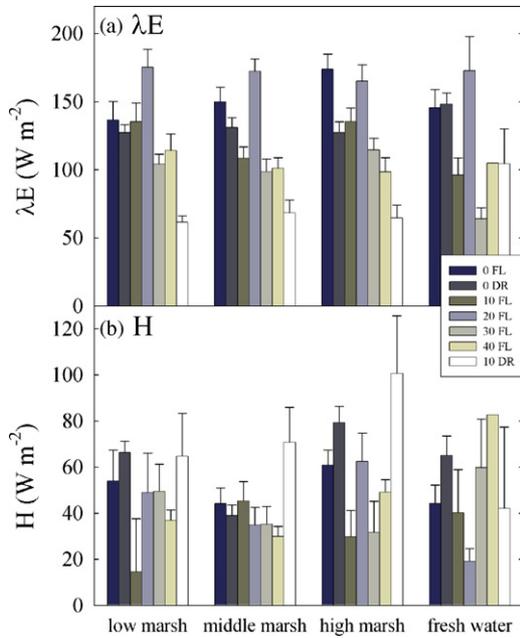


Fig. 5. The effect of decreasing soil  $\Psi$  on (a) latent heat flux of leaves ( $\lambda E$ ) and (b) sensible heat loss by leaves ( $H$ ). Bars indicate  $\pm$  S.E. ( $n=3-91$ ). Species are grouped by ecological functional type; treatments are arranged by decreasing  $\Psi$  and are labeled as in Fig. 2.

indicated increasing Bowen ratios with salinity in the marsh species.

### 3.2. Oxidative stress

Mean leaf APX activities ranged from  $10.2 \mu mol g^{-1} min^{-1}$  in 10‰ salinity flooded low marsh plants up to  $29.6 \mu mol g^{-1} min^{-1}$  in 40‰ salinity flooded high marsh plants (Fig. 7). There was a significant interaction between treatment and species grouping in APX activity (ANOVA,  $p < 0.001$ ). The middle and high marsh species significantly increased APX activities in response to decreasing  $\Psi$  (ANOVA,  $p \leq 0.044$ ), while the fresh water and low marsh species did not (ANOVA,  $p \geq 0.580$ ). Additionally, APX activities in the high marsh species were significantly higher compared to all other species groupings (ANOVA,  $p < 0.001$ ).

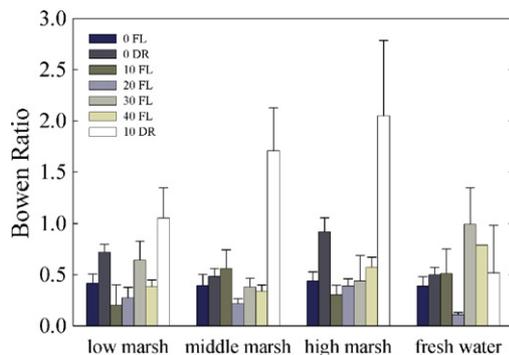


Fig. 6. The effect of decreasing soil  $\Psi$  on leaf Bowen ratios ( $H/\lambda E$ ). Bars indicate  $\pm$  S.E. ( $n=3-91$ ). Species are grouped by ecological functional type; treatments are arranged by decreasing  $\Psi$  and are labeled as in Fig. 2.

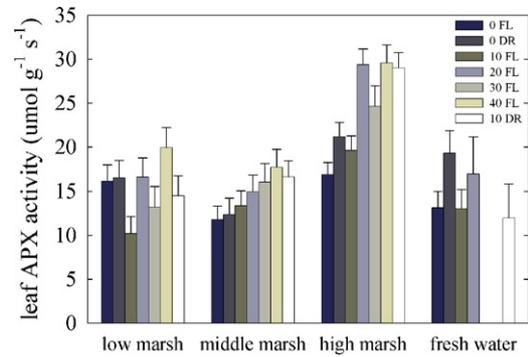


Fig. 7. The effect of decreasing soil  $\Psi$  on leaf ascorbate peroxidase (APX) activities. Bars indicate  $\pm$  S.E. ( $n=3-15$ ). Species are grouped by ecological functional type; treatments are arranged by decreasing  $\Psi$  and are labeled as in Fig. 2.

## 4. Discussion

### 4.1. Effects of decreasing water potential

Environmental conditions vary across intertidal zones in salt marshes. Plants in low intertidal marsh settings are subjected to frequent tidal inundations with brackish water. In contrast, plants in high marsh settings often experience dried soils between infrequent inundations, leading to elevated soil salinities and low water potentials. Survival in these sediments requires mechanisms to maintain adequate water relations as well as a proper balance of interception and dissipation of solar energy. The results of this study indicate that many salt marsh species are adapted to the prevailing conditions in their specific zone of the salt marsh by variations in leaf biophysics, biochemistry, and morphology.

Incoming solar radiation was efficiently absorbed by all species in the study. Leaf shortwave absorptivities ( $\alpha_s$ ) ranged from 0.62 to 0.70 and were slightly higher than expected  $\alpha_s$  values for  $C_3$  inland plants reported by Campbell and Norman (1998). The thick, dark leaves of  $C_4$  *Spartina* species may help to intercept more solar radiation compared to other herbaceous plants that may not be adapted to high light environments that are typical in salt marshes. While some plants appeared to suffer salinity-induced bleaching of leaves at high salinity, this increase in transmissivity did not lead to differences between treatments. Salt crystals were secreted on leaves under salinity treatments, leading to increased reflectance that apparently overshadowed any bleaching effects. Thus, there were no differences in  $\alpha_s$  between treatments or species.

Since the plants in this study were grown under identical conditions, all received equal amounts of radiant energy. Plants at high water potential (high  $\Psi$ ; flooded soil, low salt) lost more heat via latent cooling than plants at low  $\Psi$  (drained soil, high salt). Plants at low  $\Psi$  tended to lose more energy via radiative and sensible pathways than plants at high  $\Psi$ . During times of water stress, transpiration rates decreased, leading to decreased rates of latent cooling. With decreased latent cooling during water stress, leaf temperatures were noted to increase by as much as  $4^\circ C$ , leading to increased radiative and sensible heat losses. Mean  $T_L$

ranged from 25.9 to 30.4 °C across treatments and species groupings (Fig. 3a), similar to measurements of *Spartina alterniflora* and *S. patens* leaves by Teal and Kanwisher (1970). Interestingly, the lowest leaf temperatures were observed in moderate salinity treatments (usually 10‰ flooded treatments), likely due to high rates of latent cooling. Mean  $\lambda E$  ranged from 61.4 W m<sup>-2</sup> in water stressed plants up to 175.4 W m<sup>-2</sup> in moderate salinity plants (Fig. 5a). These measurements are slightly lower than maximum values reported by Jacobs et al. (2002) for a prairie wetland and Teal and Kanwisher (1970) for *S. alterniflora* and *S. patens* in field settings. These differences are likely due to the conditions under which measurements were taken. The greenhouse conditions in the present study had a lower radiation load than the field sites used by Jacobs et al. (2002) and Teal and Kanwisher (1970). A higher radiation load under similar temperatures likely led to increased rates of latent heat flux in these studies (Eq. (12)) compared to the present study.

Latent cooling appeared to be similar across all ecological functional types, but sensible heat loss ( $H$ ) became more important in water-stressed high marsh species compared to low marsh species. Plants with low transpiration rates in low  $\Psi$  treatments may be adapted to tolerate higher leaf temperatures in times of low latent heat loss. Indeed, rates of latent heat loss complemented rates of  $H$  for many plants in this study. Leaf dimensions can be important when considering leaf boundary layer conductances to heat ( $g_{Ha}$ ; Eq. (11)) and water vapor ( $g_{va}$ ; Eq. (17)). Mean  $H$  ranged from 14.5 to 100.6 W m<sup>-2</sup> across treatments (Fig. 5b). These measurements are slightly lower than values reported by Jacobs et al. (2002) for a prairie wetland but similar to the values of Teal and Kanwisher (1970) for *S. alterniflora* and *S. patens* in field settings.  $H$  is proportional to the difference in temperature between leaf and air (Eq. (10); Campbell and Norman, 1998), and increased with decreasing soil  $\Psi$ . Similarly, Souch et al. (1998) found  $H$  to increase with drying soil conditions on lakeshore communities. Additionally, the high marsh species in this study showed significantly higher rates of sensible heat loss compared to middle and low marsh species. High marsh conditions usually involve high salinity regimes and dry soil between infrequent tidal fluxes. Therefore, plants adapted to life in these conditions may be adapted to withstand higher leaf temperatures, allowing higher rates of sensible heat flux to conserve water in times of low latent cooling. The high-marsh species have narrow leaves (Fig. 3b) that help to increase conductance to heat loss ( $g_{Ha}$ ; Eq. (11)) and therefore help increase  $H$  (Eq. (10)). Narrow leaves also increase the conductance to water loss (Eq. (17)), but many similar plants are noted to curl leaves or orient leaves paraheliotropically to reduce water loss (Heckathorn and DeLucia, 1991).

Leaf size, best represented by characteristic dimension ( $d$ ), is therefore of interest when considering responses of plants to conditions that increase  $T_L$ . Growth of many plants became stunted under low  $\Psi$  conditions, which may indicate sensitivity, but may also provide increased ability to dissipate heat. Significantly increasing Bowen ratios in the middle and high marsh species suggest these species are adapted to tolerate higher leaf temperatures, resulting in higher rates of sensible heat loss, compared to the freshwater species.

Mean stomatal conductance ( $g_s$ ) measures ranged from 49 mmol m<sup>-2</sup> s<sup>-1</sup> in low  $\Psi$  treatments up to 206 mmol m<sup>-2</sup> s<sup>-1</sup> in high  $\Psi$  treatments, similar to results published for *S. pectinata* by Heckathorn and DeLucia (1991). The decreased leaf vapor conductance with decreased  $\Psi$  also resulted in a decreased leaf conductance to CO<sub>2</sub> ( $g_{CO_2}$ ; Fig. 4). This will lead to a decrease in CO<sub>2</sub> assimilation rates ( $A$ ), consistent with observations of decreased photosynthetic rates under salinity in many other salt marsh species (reviewed by Drake, 1989). If one assumed ambient CO<sub>2</sub> concentrations of 370 ppm and  $C_i/C_a$  ratios 0.27–0.36 (Henderson et al., 1998), values for  $A$  in this study would be very similar to previously published measures of CO<sub>2</sub> uptake by *S. pectinata* under salinity stress (Heckathorn and DeLucia, 1991).

It appears that energy may be gained and lost by different means with the onset of drought or salinity stress in marsh halophytes. The results of this study suggest both drought and salinity have similar effects on marsh halophytes. Moreover, none of these results may be directly attributable to salt ion toxicity. In these marsh halophytes, the major influence of salt appears to be lowering soil  $\Psi$ , although increasing APX activities may indicate oxidative stress also contributes to decreased marsh production with increasing salinity. However, stomatal conductance (Fig. 4) and  $\lambda E$  (Fig. 5) were lower in drained treatment plants (both 0‰ and 10‰ salinity treatments) than would be expected solely from a  $\Psi$  gradient. Environmental factors in addition to  $\Psi$  may exist in drained soil conditions that may yet need to be addressed. The role of toxic ion effects in these salinity treatments may be an interesting area for future investigation.

#### 4.2. Oxidative stress

In the present study, leaf APX activities ranged from 10.2  $\mu\text{mol g}^{-1} \text{min}^{-1}$  in 10‰ salinity flooded low marsh plants up to 29.6  $\mu\text{mol g}^{-1} \text{min}^{-1}$  in 40‰ salinity flooded high marsh plants (Fig. 7). These values are lower than APX activities published previously for cotton (Gossett et al., 1996), tomato species (Shalata et al., 2001), and lotus (Ushimaru et al., 2001), but more similar to values published for potato (Benavides et al., 2000), maize (Jahnke et al., 1991), and rice (Lee et al., 2001). Previous studies have shown APX activities to increase with increasing salinity in rice (Lee et al., 2001) and the salt-tolerant tomato species *Lycopersicon pennellii* (Shalata et al., 2001; Mittova et al., 2004). In the present study, APX activities were found to increase in the middle and high marsh species groupings. This indicates increasing salt or drought stress led to over-reduction of photosynthetic electron carriers, leading to the formation of reactive oxygen species (Asada, 1999). High antioxidant activities may be one way high marsh species are adapted for the highly saline conditions found in high marsh areas.

#### 5. Conclusions

The marsh plants in this study appeared to perform best under moderate salinity levels (10–20‰). Stomatal conductance and  $\lambda E$  were at their highest and  $T_L$  was at its lowest under moderate salinity. Additionally, maximum growth occurred at 10–20‰

salinity (data not shown). Many of the effects of salt on plants come as a result of water stress. Tolerance to saline soils appears to be due in part to specific biophysical, morphological, and biochemical adaptations in plants. The narrow leaves characteristic of high marsh species may be an adaptation to help regulate leaf temperature in times of low latent cooling. Further work may be needed to characterize changes in halophyte leaf size in response to environmental salinity. Additionally, the high marsh species had higher APX activities compared to the other species in the study, indicating an increased ability to resist oxidative stress. These strategies may be adaptations for plant growth in the highly saline upper marsh zones. This indicates that salt marsh *Spartina* and *Distichlis* species are highly tolerant of saline soils, since growth is enhanced under moderate salinity (Greenway and Munns, 1980).

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