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Effects of salinity on chlorophyll fluorescence and CO_2 fixation in C_4 estuarine grasses

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Abstract

The effects of salinity (sea water at 0 ‰ *versus* 30 ‰) on gross rates of O_2 evolution (J_{O2}) and net rates of CO_2 uptake (P_N) were measured in the halotolerant estuarine C_4 grasses *Spartina patens*, *S. alterniflora*, *S. densiflora*, and *Distichlis spicata* in controlled growth environments. Under high irradiance, salinity had no significant effect on the intercellular to ambient CO_2 concentration ratio (C_i/C_a). However, during photosynthesis under limiting irradiance, the maximum quantum efficiency of CO_2 fixation decreased under salinity across species, suggesting there is increased leakage of the CO_2 delivered to the bundle sheath cells by the C_4 pump. Growth under salinity did not affect the maximum intrinsic efficiency of photosystem 2, PS2 (F_V/F_M) in these species, suggesting salinity had no effect on photosynthesis by inactivation of PS2 reaction centers. Under saline conditions and high irradiance, P_N was reduced by 75 % in *Spartina patens* and *S. alterniflora*, whereas salinity had no effect on P_N in *S. densiflora* or *D. spicata*. This inhibition of P_N in *S. patens* and *S. alterniflora* was not due to an effect on stomatal conductance since the ratio of C_i/C_a did not decrease under saline conditions. In growth with and without salt, P_N was saturated at ~500 μmol(quantum) m^{-2} s^{-1} while J_{O2} continued to increase up to full sunlight, indicating that carbon assimilation was not tightly coupled to photochemistry in these halophytic species. This increase in alternative electron flow under high irradiance might be an inherent function in these halophytes for dissipating excess energy.

Additional key words: *Distichlis spicata*; gas exchange; net photosynthetic rate; salt stress; *Spartina* species; species differences; stomatal conductance.

Introduction

Coastal salt marshes are inundated twice daily with tides containing moderate to high salinity levels (Pennings and Bertness 2001). Porewater salinities in salt marshes are normally slightly brackish but can range up to levels greater than the 32–35 ‰ salinity of adjacent ocean water. C_4 grasses tend to dominate many of these habitats, most notably grasses of the genus *Spartina* (Poaceae). *Spartina* flourishes in saline estuarine conditions and exhibits rapid biomass production (Long and Woolhouse 1979). Annual net productivity for stands of *S. alterniflora* Loisel. in Georgia can be as high as 3 990 g m^{-2} (Odum and Fanning 1973). The photosynthetic characteristics of salt marsh grasses are highly relevant to understanding primary productivity in one of the most productive ecosystems in the biosphere.

Salt ion toxicity has numerous deleterious effects on

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Abbreviations: C_i/C_a – ratio of intercellular to ambient CO_2 concentrations; Chl – chlorophyll; ETR – electron transport rate through PS2; F_0 – minimal fluorescence from PS2 following dark adaptation; F_M – maximum fluorescence yield from PS2 following saturating pulse of photons in a dark-adapted plant; F_V – variable fluorescence = $F_M - F_0$; F_S – steady-state yield of PS2 fluorescence in the light; F_M' – maximum fluorescence yield from PS2 following a saturating pulse of photons in a light-adapted plant; g_s – stomatal conductance; J_{O2} – gross rate of O_2 evolution from PS2 calculated from fluorescence parameters; NPQ – non-photochemical quenching, calculated as $(F_M - F_M')/F_M'$; P_N – net photosynthesis rate; $P_N^* = P_N + R_D$ – gross photosynthesis rate corrected for dark respiration rate; PPFD – photosynthetic photon flux density (400–700 nm); PS2 – photosystem 2; Φ_{PN} – maximum quantum efficiency of CO_2 fixation measured under limiting light; Φ_{JO2} – maximum quantum efficiency of O_2 evolution measured under limiting irradiance; Φ_{PS2} – yield of PS2, calculated as $(F_M' - F_S)/F_M'$.

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plants such as denaturing cytosolic enzymes and facilitating the formation of reactive oxygen species that can damage membranes and proteins (reviewed by Zhu 2001). Additionally, stomatal conductance (g_s) can be reduced with increasing environmental salinity. Like drought stress, salt stress involves a decrease in soil water potential. Stomates are sensitive to changes in soil water potential and will usually close during times of drought and salt stress (Willmer 1983). A decrease in g_s reduces incoming CO_2 and thus can reduce photosynthesis rates.

Studies of the effects of salinity on carbon assimilation in C_4 photosynthesis can enable a more comprehensive understanding of productivity in C_4-dominated salt marshes. Previous studies have investigated isotopic influences of salinity on CO_2 fixation in some C_3 species (*e.g.* Farquhar *et al.* 1982, Neales *et al.* 1983, Brugnoli and Lauteri 1991, van Groenigen and van Kessel 2002) but only in a very few C_4 species (Bowman *et al.* 1989, Meinzer *et al.* 1994, Sandquist and Ehleringer 1995, Zhu and Meinzer 1999, Maricle and Lee 2006). However, it remains unknown how salt-tolerant C_4 species differ from salt-sensitive C_4 species in terms of carbon assimilation and usage of solar energy in photosynthesis.

CO_2 fixation rates in salt marsh plants are sensitive to increasing salinity (reviewed by Drake 1989), but how are light-harvesting processes affected by salt stress? Many previous studies have investigated the effects of salt on chlorophyll (Chl) fluorescence parameters like F_V/F_M, Φ_{PS2}, photochemical quenching, NPQ, ETR, F_0, and F_M in both salt-sensitive and salt-tolerant species (*e.g.* Mishra *et al.* 1991, Brugnoli and Björkman 1992, Jimenez *et al.* 1997, Nieva *et al.* 1999, Castillo *et al.* 2000, Misra *et al.* 2001, James *et al.* 2002, Lu *et al.* 2002, Morant-Manceau *et al.* 2004, Naidoo and Kift 2006, Ranjbarfordoei *et al.* 2006, Sixto *et al.* 2006). While there does not appear to be a strong relationship between fluorescence parameters and salt sensitivity, some of these studies suggest that photochemistry in halophytes may be more resistant to salt stress compared to glycophytes. However, the effects of salinity on the combination of light harvesting and CO_2 fixation remain less clear because few studies have investigated both processes. This is why we simultaneously measured gross rates of O_2 evolution from PS2 (J_{O2}) by Chl fluorescence and rates of CO_2 fixation (P_N) in C_4 salt marsh grasses grown under fresh water as well as salinity levels higher than those normally prevailing in their natural habitat.

Materials and methods

Spartina alterniflora was collected in Willapa Bay, Washington (46°35'N, 124°01'W). *S. patens* (Aiton) Muhl. was obtained from the Gulf of Mexico near Panacea, Florida (30°02'N, 84°23'W). *S. densiflora* Brongn. plants were obtained from the Odiel salt marshes, SW Spain (37°17'N, 06°55'W). *Distichlis spicata* (L.) Greene was collected in southern Skagit Bay, Washington (48°15'N, 122°26'W). Individual tillers from all plants were potted in 11×11 cm pots using a 50/50 (v/v) sand/potting soil mixture and were watered to saturation twice weekly with modified Hoagland nutrient solution (Epstein 1972). Potted plants were allowed 30 d to recover before initiating experimental treatments.

Photosynthetic parameters were measured in two separate sets of plants; the first set was grown in a growth chamber and the second set was grown in a greenhouse. Plants were randomized between treatments (drained *vs.* flooded soil; 0, 15, or 30 ‰ salt). Drained treatment plants were watered to saturation twice weekly with nutrient solution containing 0 ‰ salt. Flooded treatment plants were placed into 50×36 cm plastic tubs. Twelve pots were placed into each tub in an unbalanced block design. Flooded treatments contained enough water to submerge plants to a level 2 cm above the soil surface (about 1 200 cm^3) and the water was replaced weekly. During the acclimation period, salinity levels were increased 10 ‰ per week until flooded treatments included 0, 15, and 30 ‰ salt in the first set of plants and 0 and 30 ‰ salt in the second set of plants (*Instant Ocean* salts containing natural levels of Na^+, Mg^{+2}, Ca^{+2}, K^+, Sr^{+2}, Cl^-, SO_4^{-2}, BO_3^{-3}, and CO_3^{-2}; *Aquarium Systems*, Mentor, OH, USA). Once proper experimental salinity levels were reached, plants grew 30–45 d in their respective treatment before measurements were made. The treatment duration was long enough to allow plants to acclimate to treatment conditions but was short enough so that plants did not become limited by pot size.

Chl fluorescence was measured in the first set of plants, grown in a walk-in growth chamber. Growth chamber conditions consisted of a 14 h photoperiod with 26 °C days and 18 °C nights. Light was provided by 400 W metal halide lamps with a PPFD of ~500 μmol m^{-2} s^{-1} near the tops of leaves. Chl fluorescence was measured in these plants with an *OS-500* modulated fluorometer (*Opti-Sciences*, Tyngsboro, MA, USA). Gross rates of photosynthetic O_2 evolution were calculated from PS2 fluorescence yield measurements after Krall and Edwards (1992). The fluorescence yield ratio Φ_{PS2}, calculated as $(F_M' - F_S)/F_M'$ (Genty *et al.* 1989), was multiplied by the PPFD, then by 0.84 (the approximate amount of incident radiation absorbed by the leaf, Björkman and Demmig 1987), then by 0.5 (assuming *ca.* half of photons are absorbed by PS2), giving the electron transport rate (ETR) per leaf area. The ETR was divided by 4 (4 electrons transported per O_2 evolved) to arrive at an estimate of the gross photosynthesis rate (J_{O2}) [μmol(O_2 evolved) m^{-2} s^{-1}]. Irradiance response curves were generated for the second-youngest leaf on each plant under incident PPFD from 15–2 000 μmol m^{-2} s^{-1}. These data were transformed using a log(J_{O2}+1) transformation and then comparisons were made between irradiance-response curves using repeated measures' analysis of covariance (ANCOVAR). In this statistical model, individual plants were the repeated effect, tubs containing multiple plants were a random effect (treatments were blocked by tub), and PPFD was the covariate (*SAS* version *8.0*, 2001 *SAS Institute*, Cary, NC, USA; α=0.05). Although this was a linear model, normally-distributed residuals indicated the irradiance-response curves were a sufficient fit. Φ_{PS2} measures were compared using similar ANCOVAR analyses. In these models, individual plants were the repeated effect, treatments were blocked by tub, and PPFD was the covariate (*SAS* version *8.0*; α=0.05).

P_N and J_{O2} were then measured in a subsequent set of greenhouse-grown plants. Greenhouse conditions included natural lighting (average PPFD was 450 μmol m^{-2} s^{-1} during daylight hours with a peak around 1 400 μmol m^{-2} s^{-1} on sunny days) and 26 °C temperatures during daytime and 18 °C during nighttime. A *FastEst* gas exchange system (Tartu, Estonia, described by Laisk and Edwards 1998) was used to measure leaf gas exchange and Chl fluorescence in these plants. Intact plant leaves were enclosed within a leaf chamber at 25 °C and 25 % relative humidity. CO_2 flow into the chamber was 360 g m^{-3} and O_2 concentration was 21 %. The *FastEst* system used a *Li-Cor 6251* CO_2 analyzer (Lincoln, NE, USA) to measure rates of CO_2 fixation and psychrometers to measure H_2O transpiration using an open differential gas exchange chamber. Measures of CO_2 uptake (P_N) and external water vapor concentrations allowed calculations of g_s and C_i/C_a. Chl fluorescence was measured simultaneously with a pulse amplitude modulated fluorometer (*Walz PAM 101*; Effeltrich, Germany), allowing comparisons of J_{O2} and P_N. Irradiance-response curves were generated for incident PPFD of 0–1 000 μmol m^{-2} s^{-1}. A 20 min dark-adaptation period was allowed before measurements at 0 μmol m^{-2} s^{-1}. The response of J_{O2} to increasing irradiance and the maximum quantum yields for O_2 evolution under limiting irradiance (Φ_{JO2}; measured by fluorescence analysis) were very similar between greenhouse and growth chamber plants.

Measurements of the initial slopes (PPFD from 0 to 100 μmol m^{-2} s^{-1}) of J_{O2} and P_N were used to calculate the quantum efficiencies of photon usage. Non-photochemical fluorescence quenching (NPQ) at 1 000 μmol m^{-2} s^{-1} was calculated after Bilger and Björkman (1990) as $(F_M - F_M')/F_M'$. Comparisons of g_s, C_i/C_a, Φ_{JO2}, Φ_{PN}, NPQ, and F_V/F_M were made between species and treatments using analysis of variance (ANOVA; *SAS* version *8.0*; α=0.05). In these models, treatments were blocked by tub.

Results

Φ_{PS2}, calculated as $(F_M' - F_S)/F_M'$, was as high as 0.74 at low irradiance and decreased with increasing irradiance in all species until 500–1 000 μmol m^{-2} s^{-1} and then remained constant near 0.35–0.40 (data not shown). There were no significant differences in Φ_{PS2} between species, treatments with or without salt, or their interactions (ANCOVAR, $p \geq 0.439$).

The maintenance of a relatively high Φ_{PS2} with increasing irradiance led to a linear increase in J_{O2} from 300 up to 2 000 μmol m^{-2} s^{-1} (Fig. 1). J_{O2} was calculated as described with inputs of Φ_{PS2} (using Chl fluorescence analysis as a measure of gross rates of O_2 evolution), which also showed little or no difference with or without salt. Maximum rates of J_{O2} were quite high in this study,

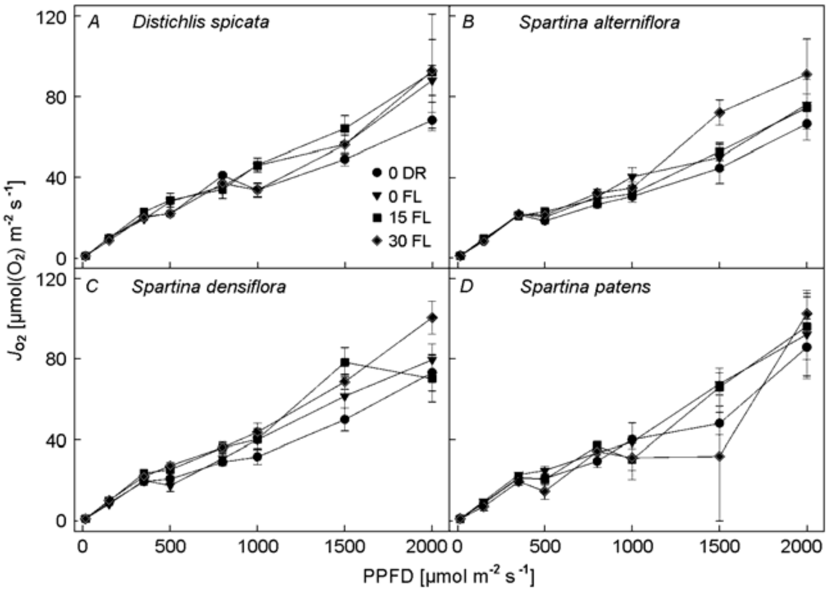

Fig. 1. Irradiance-response curves showing calculated J_{O2} in the first set of *Spartina* and *Distichlis* plants, grown in the growth chamber. Plants were maintained in flooded or drained soil conditions as well as salt up to 30 ‰. Means of 3–14 plants ± SE are shown. The number (0, 15, or 30) represents the treatment salinity [‰] and DR or FL indicates drained or flooded soil conditions.

consistent with high light-harvesting capacities typical of C_4 plants (Long 1999). Rates of J_{O2} were not different between the two sets of plants (growth chamber grown: Fig. 1; greenhouse grown: Fig. 2). There were no significant differences in J_{O2} between any species, salinity treatments, or growing location (ANOVA, $p \geq 0.249$).

Maximum quantum efficiencies of Φ_{JO2}, measured under limiting irradiance, were not significantly decreased by increased salinity in any species (Tables 1 and 2). In growth chamber plants, mean Φ_{JO2} ranged from 0.054–0.067 O_2 per photon (Table 1). In greenhouse plants, mean Φ_{JO2} ranged from 0.046–0.068 O_2 per photon (Table 2). There were no significant differences in Φ_{JO2} between species, treatments, or growing location (ANOVA, $p \geq 0.298$).

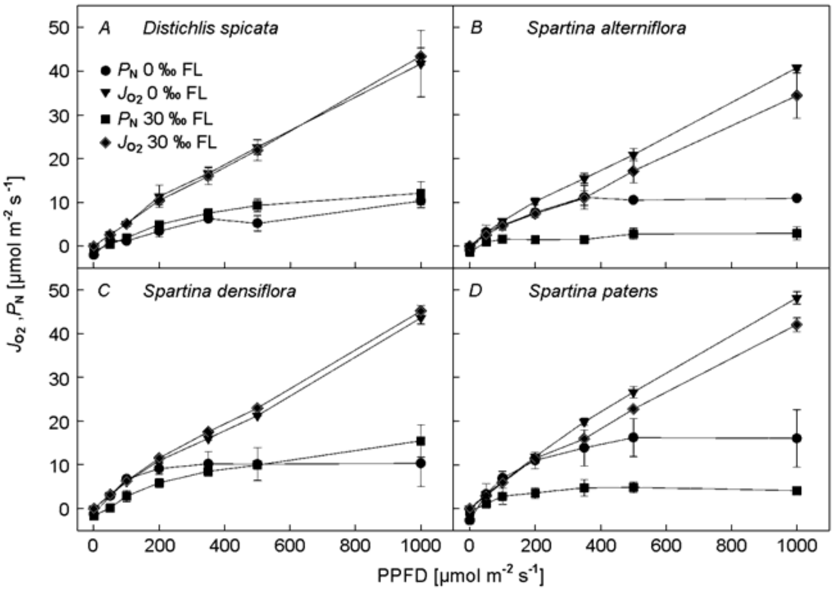

Fig. 2. Irradiance-response curves showing J_{O2} and P_N in the second set of *Spartina* and *Distichlis* plants, grown in the greenhouse. Plants were maintained under flooded soil conditions at 0 or 30 ‰ salt. Means of 3 plants ± SE are shown. Symbols are labeled by treatment (as in Fig. 1).

Table 1. Maximum Φ_{JO2} [O_2 photon^{-1}] measured under limiting irradiance (PPFD 0–100 µmol m^{-2} s^{-1}) in *Spartina* species and *D. spicata* in the first set of plants, grown in the growth chamber. The mean ± SE (n) is shown for each species and treatment combination. Treatment labels express the salinity [‰], and "drained" or "flooded" indicates the waterlogging treatment in which the plants were grown.

	0 ‰ drained	0 ‰ flooded	15 ‰ flooded	30 ‰ flooded
S. alterniflora	0.061 ± 0.002 (14)	0.062 ± 0.003 (8)	0.059 ± 0.003 (10)	0.062 ± 0.003 (8)
S. densiflora	0.060 ± 0.003 (7)	0.057 ± 0.001 (4)	0.067 ± 0.001 (6)	0.062 ± 0.002 (4)
S. patens	0.060 ± 0.002 (6)	0.065 ± 0.002 (3)	0.061 ± 0.003 (4)	0.056 ± 0.005 (3)
D. spicata	0.058 ± 0.002 (6)	0.054 ± 0.002 (4)	0.065 ± 0.003 (3)	0.058 ± 0.004 (5)

In the study comparing the efficiencies and rates of P_N and J_{O2}, values of Φ_{PN} measured under limiting irradiance were lower than Φ_{JO2} (Table 2). Values for Φ_{PN} ranged from 0.029–0.061 CO_2 per photon. Φ_{PN} significantly decreased with increased salinity across species (ANOVA, $p < 0.001$). There were no significant differences in Φ_{PN} between species (ANOVA, $p = 0.112$). Large differences were observed between J_{O2} and P_N, especially at high irradiance (Fig. 2; paired *t*-test, $p < 0.001$). P_N values at 1 000 µmol m^{-2} s^{-1} were lower in the 30 ‰ salinity compared to the 0 ‰ salinity in *S. alterniflora* and *S. patens* (ANOVA, $p \leq 0.063$) but not in *S. densiflora* or *D. spicata* (ANOVA, $p \geq 0.636$). Values of g_s at 1 000 µmol m^{-2} s^{-1} ranged 0.020–0.099 mol m^{-2} s^{-1} (Table 2). In a manner similar to P_N (Fig. 2), at high irradiance g_s significantly decreased with increasing salinity in *S. alterniflora* and *S. patens* (ANOVA, $p \leq 0.032$) but not in *S. densiflora* or *D. spicata* (ANOVA, $p \geq 0.478$). At PPFD of 1 000 µmol m^{-2} s^{-1} the mean value for C_i/C_a across species and treatments was 0.53 (Table 2). Values for C_i/C_a did not differ between species and did not change in response to salinity (ANOVA, $p \geq 0.478$).

Since J_{O2} increased linearly while P_N was at a plateau at high irradiance, a linear relation was not observed in these plants when rates of J_{O2} were compared to P_N^* (Fig. 3). This hyperbolic shape became especially noticeable in *S. alterniflora* and *S. patens*, where rates of P_N were significantly decreased by salinity.

Decreased P_N with unchanged J_{O2} (*e.g.* Figs. 2 and 3) indicate an increase in sinks other than CO_2 fixation for the flux of electrons through photochemistry. We found

Table 2. Photosynthesis data collected in the gas exchange system for the second set of *Spartina* and *Distichlis* plants, grown in the greenhouse. Plants were maintained under flooded conditions containing 0 or 30 ‰ salinity. Shown are the maximum net quantum efficiency of CO_2 fixation (Φ_{PN}) and the gross quantum efficiency of O_2 evolution (Φ_{JO2}) under limiting irradiance (PPFD 0–100 µmol m^{-2} s^{-1}). The leaf conductance to CO_2 (g_s), the C_i/C_a, and the maximum amount of non-photochemical quenching (NPQ) were measured at 1 000 µmol(quantum) m^{-2} s^{-1}. The maximum F_V/F_M of plants was measured following 20 min of dark adaptation. The mean ± SE of three replicates is given for each species/treatment combination. *Letters in parentheses* represent significant differences at α=0.05 (Tukey's pairwise comparison).

Species	Treatment salinity [‰]	Φ_{PN} [CO_2 $photon^{-1}$]	Φ_{JO2} [O_2 $photon^{-1}$]	g_s [mol m^{-2} s^{-1}]	C_i/C_a	Max NPQ	Max F_V/F_M
S. alterniflora	0	0.058±0.010 (a)	0.066±0.006 (a)	0.063±0.001 (a)	0.53±0.06 (a)	1.99±0.14 (a)	0.74±0.01 (a)
	30	0.035±0.002 (b)	0.046±0.006 (a)	0.020±0.005 (b)	0.60±0.09 (a)	1.80±0.21 (a)	0.67±0.05 (a)
S. densiflora	0	0.059±0.007 (a)	0.056±0.007 (a)	0.065±0.016 (a)	0.57±0.09 (a)	1.78±0.12 (a)	0.74±0.01 (a)
	30	0.037±0.011 (b)	0.063±0.003 (a)	0.064±0.004 (a)	0.35±0.07 (a)	1.44±0.06 (ab)	0.73±0.01 (a)
S. patens	0	0.059±0.012 (a)	0.068±0.002 (a)	0.099±0.019 (a)	0.56±0.08 (a)	1.19±0.05 (b)	0.71±0.01 (a)
	30	0.029±0.011 (b)	0.057±0.004 (a)	0.024±0.002 (b)	0.53±0.11 (a)	1.70±0.67 (a)	0.72±0.01 (a)
D. spicata	0	0.039±0.006 (b)	0.050±0.008 (a)	0.059±0.004 (a)	0.53±0.02 (a)	0.79±0.13 (b)	0.60±0.06 (a)
	30	0.030±0.003 (b)	0.057±0.003 (a)	0.069±0.008 (a)	0.53±0.11 (a)	1.39±0.19 (a)	0.69±0.02 (a)

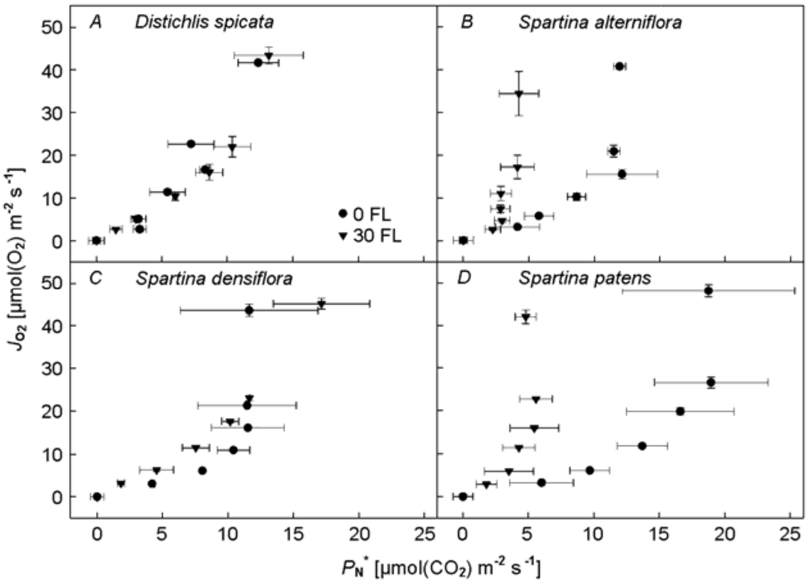

Fig. 3. The relationship between J_{O2} and P_N^* in the second set of *Spartina* and *Distichlis* plants, grown in the greenhouse. Plants were maintained under flooded soil conditions at 0 or 30 ‰ salt. Means of 3 plants ± SE. Symbols indicate whether the plants were grown under 0 ‰ (●) or 30 ‰ salt (▼).

higher NPQ with increasing salinity in *S. patens* and *D. spicata* (ANOVA, $p \le 0.073$), but not in *S. alterniflora* or *S. densiflora* (Table 2; ANOVA, $p \ge 0.570$). Additionally, dark-adapted F_V/F_M as a measure of photoinhibition did not differ significantly between salinity treatments in any species (Table 2; ANOVA, $p \ge 0.817$).

Discussion

Our results suggest some halotolerant species may be adapted to constitutively dissipate excess photon energy by electron sinks other than CO_2 fixation. In C_4 plants a number of studies have shown a close correlation between gross rates of O_2 evolution from PS2 (J_{O2}) measured by Chl fluorescence and P_N^* in response to varying irradiance (Genty *et al.* 1989, Oberhuber *et al.* 1993, Earl and Tollenaar 1998), CO_2 (Krall and Edwards 1990, Edwards and Baker 1993), or temperature (Oberhuber and Edwards 1993, Naidu and Long 2004). However, there are also some reports of changes in the ratio of J_{O2} to P_N. Lal and Edwards (1996) measured an increase in J_{O2}/P_N^* with decreasing relative water content in maize leaves. Earl and Tollenaar (1998) observed an increase in the proportion of J_{O2} later in development after grain filling in maize. Under low temperatures there is a clear increase in PS2 activity per CO_2 fixed in maize, a chilling sensitive species (Fryer *et al.* 1998). This was suggested to be due to an increase in the Mehler reaction with increased scavenging of reactive oxygen species. Recently, in two chilling tolerant C_4 species, *Cyperus longus* L. and *Miscanthus×giganteus* (Greef & Deuter *ex* Hodkinson & Renvoize) the ratio of Φ_{PS2}/Φ_{PN}^* increased when grown under low temperature (Farage *et al.* 2006). Similarly, Φ_{PS2}/Φ_{PN}^* increased with decreasing assay temperature in *Flaveria bidentis* L. Kuntze (Kubien *et al.*

2003). Moreover, seasonal variations in the ratio of PS2 activity to P_N were observed in *Spartina alterniflora* (Baerlocher *et al.* 2004). We found that increasing irradiance resulted in an increase in J_{O2}/P_N^* in all species, and increasing salinity increased J_{O2}/P_N^* in *S. alterniflora* and *S. patens*.

An increase in soil salinity decreases CO_2 fixation rates, but reports on the effects of salinity on light-harvesting have been inconclusive. We found little or no effects on Φ_{PS2} in any species (data not shown). Therefore, the J_{O2} values calculated from Φ_{PS2} were not changed by salinity (Figs. 1 and 2). Also F_V/F_M did not change with salinity in any species (Table 2). Unaffected photochemistry under increasing salinity may suggest a higher tolerance to salt in these marsh halophytes compared to salt sensitive plants. Previous studies have shown photochemistry to be more resistant to salt stress in more halotolerant species like *Suaeda salsa* L. or salt-tolerant lines of *Triticum turgidum* L. (James *et al.* 2002, Lu *et al.* 2002). Salt stress increased F_0 in salt sensitive species like *Vigna radiata* L. or *Brassica juncea* Coss. (Misra *et al.* 2001), potentially due to strong limitations on photochemical quenching in the light (*i.e.* use of energy in photochemistry) that can lead to photoinhibition as indicated by low F_V/F_M values measured in dark-adapted material (Bolhàr-Nordenkampf and Öquist 1993).

Our study suggests biophysical light-harvesting processes are much less sensitive to salinity than carbon assimilation processes. Leaf Chl contents did not change with increasing salinity (ANOVA, p=0.250; Maricle and Lee, unpublished), suggesting light-harvesting capacities are similar across salt treatments. Increasing salinity had no effect on J_{O2} in any species but decreased P_N in *S. alterniflora* and *S. patens*. J_{O2} was higher than P_N in all species (Fig. 2) which is expected due to respiratory losses of CO_2 and alternative electron sinks (*e.g.* N assimilation). The values of P_N were close to many of the laboratory-measured photosynthesis rates of estuarine species reviewed by Drake (1989). P_N was not inhibited by salt in *S. densiflora* or *D. spicata* plants in this study. This result contrasts with published gas exchange rates for a number of other estuarine grasses exposed to salinity (Drake 1989), which may suggest a superior level of salt tolerance in these two species.

Salinity effects under limiting irradiance can be assessed by quantum efficiency measurements. Values for Φ_{JO2} in *Spartina* and *Distichlis* were slightly lower than values of quantum yields of O_2 evolution (measured with an O_2 electrode) reported by Lal and Edwards (1995) for several C_4 species under saturating CO_2. Values for Φ_{JO2} were not changed by salinity in any species (Tables 1 and 2). Values of Φ_{PN} were generally much lower than Φ_{JO2}, and were significantly reduced under 30 ‰ salinity across species (Table 2). Decreases in Φ_{PN} were not coupled to photochemistry, since Φ_{JO2} values were unchanged. Thus, salt-induced decreases in Φ_{PN} under limiting irradiance may be explained by increases in bundle sheath leakage of CO_2. Carbon isotope discrimination increases by as much as 2 ‰ in these species in response to salinity (Maricle and Lee 2006) which could occur by increased bundle sheath leakage of CO_2 or a decrease in C_i/C_a. The C_i/C_a ratio measured under high irradiance did not significantly decrease under salinity (Table 2). Thus, decreases in Φ_{PN} under salinity with unchanging C_i/C_a suggest increasing bundle sheath CO_2 leakage, which would result in additional photochemistry per CO_2 fixed. This is consistent with studies by Bowman *et al.* (1989) and Meinzer *et al.* (1994) that showed bundle sheath CO_2 leakage increased in response to salinity in C_4 species.

At high irradiance, high photochemistry rates occurred with substantially lower CO_2 fixation rates (Fig. 2). This difference is unusual, since gross and net photosynthetic processes are tightly coupled in many C_4 species (see earlier). Perhaps the uncoupling of P_N^* from J_{O2} (Fig. 3) observed in the present study is an example of how some C_4 species dissipate excess energy through photochemistry. Increases in rates of the Mehler reaction could help dissipate the surplus of energy (Earl and Tollenaar 1998); the Mehler reaction can represent a large electron sink in some cases (Farage *et al.* 2006). One factor contributing to salt tolerance may be the ability to regulate usage of captured solar energy during times of low P_N.

In our study, P_N saturated at 12–15 μmol(CO_2) m^{-2} s^{-1}, lower than values normally expected for C_4 plants, but similar to measurements by Ewing *et al.* (1995) and Nieva *et al.* (1999, 2003) for *Spartina* plants grown in greenhouses. At high irradiance, CO_2 fixation was significantly decreased by salinity in *S. alterniflora* and *S. patens*, but not in *S. densiflora* or *D. spicata* (Fig. 2). This depression in P_N coincided with a decrease in g_s with salinity in *S. alterniflora* and *S. patens* (Table 2). However, C_i/C_a was unchanged with salinity in all species (Table 2), suggesting changes in g_s are not directly responsible for regulating incoming carbon under salt stress. Unchanging C_i/C_a with decreasing g_s may also indicate that internal cycling of CO_2 increases with salinity which could have a feedback effect reducing g_s.

Since light-harvesting rates will invariably be larger than CO_2 fixation rates under high irradiance, excess energy must be safely dissipated to prevent damage of PS2 reaction centers (Demmig-Adams and Adams 1992). Decreases in the dark-adapted F_V/F_M ratios of Chl fluorescence below ~0.8 can indicate a decrease in active PS2 reaction centers (Bolhàr-Nordenkampf and Öquist 1993). The F_V/F_M ratios measured for plants in this study averaged 0.70. This could indicate some sustained inactivation of reaction centers or inadequate dark adaptation (20 min used in this study) to allow complete recovery of F_V/F_M. In this study F_V/F_M ratios were not significantly reduced by salinity in any species (Table 2), suggesting excess energy was efficiently dispersed without PS2 inactivation. Prevention of photoinhibition is important in determining plant resistance to environmental stresses that reduce carbon fixation relative to light-harvesting rates (Demmig-Adams and Adams 1992).

Brugnoli and Björkman (1992) and Nieva *et al.* (1999) found gas exchange measurements to be much more sensitive to salinity than photoinhibition at PS2 (from measurements of the dark-adapted F_V/F_M ratio of Chl fluorescence). The current study shows mechanisms are engaged that prevent photoinhibition in these halophytes. Irradiance in excess of that used in CO_2 fixation is largely dissipated through photochemistry, which may occur by the Mehler reaction, by increasing internal cycling of CO_2, and/or by photorespiration. Dissipation of excess energy by NPQ can also prevent photoinhibition. NPQ was engaged under a PPFD of 1 000 μmol m^{-2} s^{-1} in these species (Table 2), and it increased with salinity in *S. patens* and *D. spicata,* but not in *S. alterniflora* or *S. densiflora*. Increasing energy dissipation by NPQ is common in response to many types of biotic and abiotic stress (Björkman and Demmig-Adams 1995). Increases in NPQ in response to salinity were observed in the green alga *Chlorococcum* sp. (Masojidek *et al.* 2000) and salt-sensitive lines of *Triticum turgidum* (James *et al.* 2002). However, NPQ does not increase in response to salt stress in some salt-tolerant plants such as *Suaeda salsa* (Lu *et al.* 2002) or salt-hardy lines of *Triticum turgidum* (James *et al.* 2002).

Our study represents one of the few attempts to coordinate the effects of salinity on light-harvesting characteristics and carbon fixation in C_4 halophytes. It shows the value of combining analysis of photochemistry with rates of carbon assimilation. The processes responsible for harvesting solar energy are largely unaffected by increasing salinity, but the use of this energy changes with increasing irradiance and increasing salinity. Fluorometers can be used to determine how energy is used and the state of PS2, including quantum yields, rates of O_2 evolution, photoinhibition, and non-photochemical quenching. Our results indicate how photosynthesis in C_4 marsh grasses may be adapted to sediment salinity. High salinity causes some decrease in carbon assimilation. Photoprotection from excess solar energy occurs not only by non-photochemical dissipation of energy, but also by photochemical processes through alternative electron sinks.

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