Vol. 29: 111–122, 2020
https://doi.org/10.3354/ab00730

AQUATIC BIOLOGY
Aquat Biol

Published September 3

Enhanced thermotolerance of photosystem II by elevated pore-water salinity in the coastal marsh graminoid *Sporobolus pumilus*

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ABSTRACT: In coastal marsh ecosystems, high salinities, anoxic waterlogged soils, and elevated summer temperatures often promote physiological strain that results in only a few tolerant halophytic species. Although not well understood, plant physiological responses to multiple stressors can be complex and may involve intensifying or offsetting reactions. In this study, we investigated physiological responses to combined salinity and high temperature in the coastal marsh graminoid *Sporobolus pumilus* (syn. *Spartina patens*). Specifically, we considered changes in plant–water relations and Photosystem II (PSII) behavior (involving chlorophyll [chl] *a* fluorescence) in heat-shocked plants that were acclimated to different salinities (0, 15, and 30 psu). Higher salinities fostered lower stomatal conductance (g), lower leaf-water potential (Ψ_{leaf}) and lower tissue-water content (θ), as well as decreased potential quantum yield (F_v/F_m) and decreased excitation energy capture efficiencies of open reaction centers (F_v'/F_m'). Heat-shocked plants acclimated to freshwater only had decreased F_v/F_m and PSII performance index (PI_{ABS}). Interestingly, there were no changes in chl *a* fluorescent outputs in heat-shocked plants acclimated to moderate salinities, and minimal changes in plants acclimated to high salinities. Approximately 25% of the heat-shocked *S. pumilus* in freshwater revealed a K-step in their polyphasic chl *a* fluorescent transients (OJIP procedure); K-steps were not observed in salt-treated plants. This suggests that, for plants residing in freshwater, heat-shock promoted disturbances in the PSII reaction centers and, in some cases, disrupted the oxygen-evolving complex. These PSII disruptions were either absent or less intense in salinity-treated plants, indicating that acclimation to environmental salts may provide PSII thermostability in *S. pumilus*.

KEY WORDS: Photosynthesis · Chlorophyll fluorescence · Photosystem II · Heat stress · Salt stress

1. INTRODUCTION

Abiotic components in coastal marsh systems are highly stressful for most vascular plant species. Reduced and anoxic waterlogged soils with elevated summer temperatures and high environmental salinities promote selective pressures that result in the prevalence of only a few tolerant halophytic species (Ranwell 1972, Pezeshki & DeLaune 1993). Environmental salt is arguably among the most stressful of these factors as it often dictates the vegetative structure and function of coastal marsh communities (Noe & Zedler 2000, Schröder et al. 2002, Wilson et al. 2015). Indeed, vascular plant zonations within salt marshes typically reflect unique patterns of salinity, including the competitive pressures among species with different salt tolerances (Adams 1963, Pennings et al. 2005, Touchette 2006). Although classified as

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halophytes due to their relatively high tolerance to environmental salts, only a few coastal plant species are actually restricted to high saline conditions (Barbour & Davis 1970, Partridge & Wilson 1987, Touchette et al. 2019). Therefore, like glycophytes, many salt-marsh plants perform best in freshwater, and increases in environmental salts often result in greater physiological stress (Barbour & Davis 1970, Touchette et al. 2012, Li et al. 2018). In a 10 wk study involving coastal graminoids, leaf chlorosis dramatically increased in *Sporobolus pumilus* when exposed to salinities ≥15 in comparison to plants grown in freshwater (Touchette et al. 2019). Furthermore, soil salinities in coastal marshes can be highly variable, and in many cases salt marshes are most productive in areas with dilute surface waters and/or where groundwater mixing lowers overall saline conditions (Butzeck et al. 2015, Touchette et al. 2019). In coastal marshes of North Carolina (USA), for example, soil pore-waters can be highly diluted, with salinities ranging from 0.8 to 19 for high and low marsh areas, respectively (Touchette 2006).

Physiological strain associated with salt stress may include altered plant–water relations (e.g. decreased tissue water potential and lower stomatal conductance) and disruptions in photosynthesis, with increasing strain as salinity levels rise (Pearcy & Ustin 1984, Drake 1989, Touchette et al. 2009a, Salpeter et al. 2012). A non-invasive tool useful in characterizing salinity-induced perturbations in Photosystem II (PSII), and hence photosynthesis, is chlorophyll *a* (chl *a*) fluorometry (Touchette et al. 2012, Dąbrowski et al. 2016, Kalaji et al. 2018). Chl *a* fluorescence outputs help characterize physiological modifications or impairments attributed to photodamage and/or photoprotection through reversible non-photochemical quenching (Cavender-Bares & Bazzaz 2004). Common markers used in chl *a* fluorescence studies, such as potential quantum yield (F_v/F_m) and excitation energy capture efficiencies of open reaction centers (RCs) (F_v'/F_m'), have been useful in identifying changes in PSII attributable to a variety of environmental stressors including heat stress at moderately elevated temperatures (between 30 and 38°C; Lu & Zhang 2000). Another marker, the chl *a* performance index (PI_{ABS}), represents a more comprehensive metric that also employs fluorescence outputs while considering plant strain associated with photon absorption efficiency, excitation energy captured by PSII, active RC densities, and the likelihood that energy continues through photochemistry (Strasser et al. 2000, Thach et al. 2007).

Owing to the characteristically harsh abiotic conditions common to many coastal marshes, these systems provide a unique opportunity to explore how multiple environmental stressors influence the physiological behavior of resident vascular halophytes. However, plant responses to multiple stressors can be quite complex, as combined stress factors may promote intensified, overlapping, or antagonistic responses (Osmond et al. 1987, Aber et al. 2001, Lu et al. 2003). In general, stress associated with elevated salt or heat on PSII performance has been investigated in terrestrial plant species (e.g. Kalaji et al. 2014, Chen et al. 2016). Less attention has been directed towards understanding the combined effects of heat and salinity on PSII, especially in coastal marsh vegetation (Lu et al. 2003, Yan et al. 2012). This lack of attention is unfortunate, as some studies on halophytes (e.g. *Artemisia anethifolia* L. and *Suaeda salsa* L.) growing in arid and semi-arid regions with high soil salinities observed limited salt-induced PSII perturbations in comparison to glycophytes (Lu et al. 2003, Wen et al. 2005). Indeed, for these halophytes, salt-adaptation had improved thermotolerance within the PSII RCs and the oxygen-evolving complexes (Lu et al. 2003, Wen et al. 2005).

A prominent salt-tolerant species that resides along the mid- and south-Atlantic coasts of North America is *Sporobolus pumilus* (Roth) P.M. Peterson & Saarela (syn. *Spartina patens* (Aiton) Muhl.; Peterson et al. 2014). This C4 grass commonly occurs as dense monotypic stands within coastal marshes and dune swales, and has a relatively wide salinity tolerance (e.g. inhabiting both freshwater and brackish systems; Bertness 1991, Hester et al. 1996). Although its salt tolerance may differ among genotypes, studies suggest that this species can tolerate salinities greater than 45 psu (Hester et al. 1996, Salpeter et al. 2012). Despite the importance of salinity in shaping primary productivity and vegetation patterns in salt marsh systems, our understanding of how salinity modifies physiological processes of coastal halophytes remains limited (Touchette 2007, Touchette et al. 2019). Understanding how halophytes respond to and interact with environmental salts in combination with other stressors may provide new insights into coastal marsh primary productivity and species distributional patterns (Rozema et al. 1985, Touchette 2006). Therefore, the purpose of this study was to elucidate the combined effects of salinity and high temperature stress on *S. pumilus*. More specifically, we sought to determine if long-term acclimation to different salinity levels (0, 15, 30 psu; comparable to freshwater, moderate, and high salinities, respectively) would influence plant–water relations and chl *a* fluorometric assessments of PSII behavior of *S. pumilus* following a sudden heat-shock event.

2. MATERIALS AND METHODS

2.1. Culture and treatment conditions

One yr old *Sporobolus pumilus*, originally germinated and grown in freshwater, were transplanted into 0.8 l containers that were placed within 20 l microcosms (2 containers per microcosm) under controlled greenhouse conditions (seasonal temperatures varied, but typically ranged between 15 and 35°C, and relative humidity between 35 and 90%). Each container was filled with natural sediments (approximately 10 cm; sandy loam soil texture [55% sand, 19% silt, 26% clay]), and water levels were maintained at 1 to 2 cm below soil surface. Shoot densities in each container were planted at levels comparable to natural systems (between 3700 and 4000 shoots m^{-2}; Curtis et al. 1989).

Salinity treatments (n = 8 microcosms each for 15 and 30 psu) were initiated in mid-February by exchanging freshwater in randomly selected microcosms with artificial seawater (15 and 30 psu; Instant Ocean, Spectrum Brands: Na^+ and Cl^- accounted for 86% of the total ions) over a 1 wk period. This dual-container microcosm (0.8 l container within a 20 l microcosm) allowed for easy exchange and maintenance of sub-surface porewater salinities. Another set of microcosms (n = 8) remained in pre-treatment freshwater conditions (0 salinity). The 24 microcosms were arranged in a randomized complete block within the greenhouse. Because of the physiological delay in *S. pumilus* when acclimating to saline soils (Salpeter et al. 2012), all plants were maintained at their respective salinities (0, 15, or 30) for 18 mo prior to initiating heat treatments. During that period, salinities were monitored from extracted interstitial waters twice a week using a refractometer and/or conductivity meter (Model 556 MPS; YSI), and water levels were held between 1 and 2 cm below soil surface. Temperature and humidity levels within the experimental greenhouse, as previously mentioned, varied seasonally and were comparable to first-year conditions.

In mid-August, individual microcosms were randomly selected to receive heat treatments (n = 4, for each salinity level). Heat shock was applied by placing selected containers in a large laboratory oven (55 cm × 45 cm × 35 cm, internal height, width, and depth; Model 40GC; Quincy Labs) at 45°C for 60 min. The temperature and stress duration used in this study were consistent with other heat-shock experiments on vascular plants (Queitsch et al. 2000, Wahid et al. 2007, Mittler et al. 2012). Heat-treated plants were then relocated to the greenhouse and exposed to natural ambient light for 2 h prior to conducting chl *a* fluorescence measurements. During heat treatment, plants were kept in the dark to minimize potential stabilizing or damaging effects of light on PSII (according to Lu & Zhang 2000). Un-heated plants were also maintained in the dark at ambient temperatures during that same 60 min period.

2.2. Plant–water relations

Leaf relative water content (θ) was evaluated immediately before and within 60 min of heat treatments. Percent water content was determined on young, fully extended leaves according to Barrs & Weatherley (1962) as modified by Joly (1985) using the following equations:

$$\theta = (W_f - W_d) / (W_t - W_d) \times 100 \quad (1)$$

where W_f is fresh weight, W_t is turgid weight, and W_d is oven-dry weight (60°C; n = 4). Turgid weights were determined by placing leaves in 50 ml sealed vials containing de-ionized water and allowing the samples to reach full turgor in darkness. Leaf water potentials (Ψ_{leaf}) of young, fully extended leaves were measured just before and within 60 min of heat treatments using a Scholander pressure chamber (Model 1000, PMS Instrument; Scholander et al. 1965).

Duplicate stomatal conductance (*g*) measurements were determined on young, fully expanded leaves using a leaf porometer (model SC-1; Decagon Devices). This instrument uses steady-state diffusion to measure vapor flux between the leaf surface and the atmosphere. As with other plant–water parameters, leaf conductance was recorded prior to heat shock and within 60 min after treatment. The duplicate measurements were pooled into a single mean value during data analysis to avoid replication within experimental units (i.e. pseudo-replication).

2.3. Chlorophyll fluorescence

In this study, polyphasic chl *a* transients (OJIP procedure; see below) were used to quantify the flow of energy through PSII as described by Strasser & Srivastava (1995) and Strasser et al. (2000). Fast chl *a* fluorescence was measured on young, fully expanded leaves before and after heat shock using a portable fluorometer (Fluorpen 100-Max, Photon Systems Instruments). Triplicate measurements from each experimental unit were pooled prior to data

analysis to avoid replication within an experimental unit. Chlorophyll fluorescence transients were initiated by applying a continuous pulse (3000 µmol quanta m^{-2} s^{-1}) of saturating blue light (470 nm) in plants acclimated to a dark room for more than 45 min. The fluorescent signals were initially recorded at 10 µs intervals for the first 600 µs, followed by progressively longer intervals over a period of 2 s resulting in 456 individual recordings (detector wavelengths between 697 and 750 nm). For the OJIP transients, O refers to the initial fluorescence that occurred around ~50 µs, J between 2 to 3 ms, I at approximately 60 ms, and P was the peak fluorescence intensity. These chl *a* fluorescent outputs were used to evaluate PSII behavior (using the JIP-test procedure; see Table 1 for parameter definitions), including the performance index (PI_{ABS}):

$$PI_{ABS} = \left(\frac{1-(F_o/F_m)}{(M_o/V_j)}\right)\left(\frac{(F_m-F_o)}{F_o}\right)\left(\frac{(1-V_j)}{V_j}\right) \quad (2)$$

where V_j is the relative variable fluorescence at the J-step (equivalent to $[F_j - F_o]/[F_m - F_o]$; F_o is initial fluorescence, F_m is maximum fluorescence, and F_j is fluorescence at the J-step) and M_o is the initial slope of the fluorescence kinetics (Thach et al. 2007, Živčák et al. 2008). For potential quantum yield (F_v/F_m), variable fluorescence yield (F_v) was calculated as $F_m - F_o$. The efficiency of excitation energy captured by open PSII RCs (F_v'/F_m') was estimated fluorometrically by applying saturating light to light-adapted leaves (acclimated between ~1000 and 1500 µmol m^{-2} s^{-1} of natural sunlight for more than 90 min).

2.4. Statistical evaluation

A repeated measures ANOVA (salinity × heat × time; using general linear models [GLMs]), with time as the repeated-measure factor, was used to evaluate plant–water relations data (g, Ψ_{leaf}, and θ) and summative fluorescent parameters (F_v/F_m, F_v'/F_m', and PI_{ABS}). To compare fluorescent data derived from the OJIP protocol following heat shock, we used a 2-way ANOVA (salinity × heat; GLM procedure), with V_i, M_o, N, ABS/RC, and TR_o/RC (see Table 1 for definitions) log-transformed prior to statistical comparisons to satisfy normality and/or equal variance assumptions. Post hoc analyses were conducted using Tukey multiple-comparison tests when significant differences were identified by ANOVAs. All comparisons were performed using SPSS Statistics 20 software (SPSS 2011) and considered significant at $\alpha = 0.05$.

Table 1. Chlorophyll *a* fluorescence outputs and polyphasic fluorescence transient parameters (from the OJIP procedure) used in this study. Descriptions and mathematical expressions (where applicable) are included for each parameter. PSII: Photosystem II; RC: reaction center; Q_A: primary quinone electron acceptor

Parameter	Description
F_j	Chl *a* fluorescence at the J-step (2 ms)
F_m	Maximum chl *a* fluorescence
F_m'	Maximum chl *a* fluorescence in light-adapted plants
F_o	Initial chl *a* fluorescence (50 µs)
F_v	Variable chl *a* fluorescence; $F_v = F_m - F_o$
F_v'	Variable chl *a* fluorescence in light-adapted plants
F_v/F_m	Potential or maximum quantum yield of PSII; $F_v/F_m = (F_m - F_o)/F_m$
F_v'/F_m'	Efficiency of excitation energy captured by PSII open RCs
F_v/F_o	Maximum potential for primary photochemistry; $F_v/F_o = (F_m - F_o)/F_o$
M_o	Net rate of RC closure; $M_o = 4(F_{300} - F_o)/(F_m - F_o)$
N	Q_A turn-over number; $N = S_m \times M_o \times (1/V_j)$
S_m	Energy needed to close all RCs; S_m= (area between curve and F_m)/($F_m - F_o$)
V_i	Relative variable fluorescence at the I-step; $V_i = (F_i - F_o)/(F_m - F_o)$
V_j	Relative variable fluorescence at the J-step; $V_j = (F_j - F_o)/(F_m - F_o)$
ABS/RC	Absorption flux per RC; ABS/RC = M_o $(1/V_j)$ $(1/(F_v/F_m))$
ET_o/CS	Electron transport flux per excited cross section; $t = 0$; $ET_o/CS = (F_v/F_m)$ $(1 - V_j)$ (ABS/CS_o)
ET_o/RC	Initial electron transport flux per RC; $t = 0$; $ET_o/RC = M_o$ $(1/V_j)$ $(1 - V_j)$
RC/CS	Density of Q_A reducing PSII RCs; $RC/CS = (F_v/F_m)$ (V_j/M_o) F_o
TR_o	Initial maximum trapping flux ($t = 0$) when all RCs are open
TR_o/CS	Initial trapping flux per excited cross section; $t = 0$; $TR_o/CS = (F_v/F_m)$ (ABS/CS_o)
TR_o/RC	Initial trapping flux ($t = 0$) when all RCs are open; $TR_o/RC = M_o$ $(1/V_j)$

3. RESULTS

Long-term exposure to different salinities produced some expected physiological changes in *Sporobolus pumilus*. High soil salinities, for example, resulted in significant alterations in plant–water relations. Stomatal conductance (g) was significantly depressed in *S. pumilus* residing in high soil salinity (30 psu) in comparison to *S. pumilus* in freshwater ($p = 0.009$; Table 2). However, we observed no significant differences in g between moderate salinity (15) and the other 2 salinity levels (0 and 30 psu; $p > 0.095$). Prominent differences in leaf water potentials (Ψ_{leaf}) were observed between the 3 salinity levels ($p = 0.004$), wherein Ψ_{leaf} declined (more negative) as salinity level increased (Table 2). In this case, Ψ_{leaf} values were between 2 and 3 times lower in salinity-treated *S. pumilus* ($p < 0.001$). Prior to heat treatment, tissue water content (θ) was lower in salt-treated plants than in those receiving only freshwater ($p = 0.019$). Water content in salt-treated plants was typically less than 65% full turgor, whereas plants receiving freshwater ranged between 72 and 80% (Table 2; $p = 0.034$ and 0.017 for moderate- and high-salinity treatments in comparison to freshwater). Water content following heat treatment was notably higher ($p < 0.001$) and more variable, with only heat-treated high-salinity plants being significantly lower than freshwater plants ($p = 0.048$). Unlike salinity treatments, the application of heat appeared to have little effect on short-term plant–water relations. In this case, the 60 min heat shock did not significantly alter g or Ψ_{leaf} in *S. pumilus* regardless of salinity treatment. It is possible that lower stomatal conductance due to dark conditions during heat-treatments resulted in an increase in tissue water content. In this case, tissue water content was significantly different in heat-shocked plants in moderate and high salinities ($p < 0.003$; Table 2). Similarly, control (un-heated) moderate- and high-salinity plants also had elevated tissue water content during the second measurement ($p < 0.001$).

Both potential quantum yield (F_v/F_m) and efficiency of energy capture by open PSII RCs (F_v'/F_m') were influenced by soil salinities prior to heat shock ($p = 0.011$ and 0.035, respectively), where F_v'/F_m' values from 30 psu plants were significantly lower than the other 2 salinities, and F_v/F_m from 30 psu plants was lower than 15 psu (Fig. 1). Prior to treatments, F_v/F_m in plants receiving only freshwater was 0.76 ± 0.008 (SE), compared to 0.70 ± 0.028 for plants adapted to high salinities (30 psu). The decreases in F_v'/F_m' were even more pronounced with high soil salinity, where mean values were 0.62 ± 0.02 and 0.41 ± 0.04 for 0 and 30 salinities, respectively ($p < 0.001$). While soil salinity influenced both PSII metrics in *S. pumilus*, only F_v/F_m was altered following heat shock ($p = 0.041$ and 0.708 for F_v/F_m and F_v'/F_m', respectively; Fig. 1). In this case, plants receiving freshwater had significant declines in F_v/F_m following heat treatment ($p = 0.017$). Potential quantum yields in heat-treated *S. pumilus* receiving moderate to high salinities were not significantly different from the un-heated controls ($p = 0.069$ and 0.166, for 15 and 30 salinity, respectively; Fig. 1). In contrast, perhaps attributed to greater variability within this metric, the chl *a* fluorescence performance index (PI_{ABS}) was apparently not influenced by soil salinity ($p = 0.196$; Fig. 1). Moreover, while there was a trend of lower PI_{ABS} for heat-shocked plants in all salinity groups, again possibly due to higher variability, only PI_{ABS} for freshwater plants was significantly different ($p = 0.014$, 0.405, and 0.404 for 0, 15, and 30 salinity, respec-

Table 2. Plant–water relation parameters measured on *Sporobolus pumilus* before (initial) and after heat-shock treatment (post-treatment). Parameters include stomatal conductance (g), leaf-water potential (Ψ_{leaf}), and relative water content (θ) for control and heat-shocked plants pre-acclimated to different salinity treatments (0, 15, or 30 psu). Statistical relationships within initial or post-heat treatments (i.e. only within a row) are indicated by letters, where values with different letters are considered significantly different. Data are presented as means ± 1 SE ($n = 4$; $\alpha = 0.05$)

Parameter	0 psu-Control	0 psu-Heat	15 psu-Control	15 psu-Heat	30 psu-Control	30 psu-Heat
g (μmol m^{-2} s^{-1})						
Initial	19.5 ± 0.9^{A}	27.9 ± 6.7^{A}	18.2 ± 1.2^{AB}	21.4 ± 7.9^{AB}	12.7 ± 3.2^{B}	10.0 ± 0.7^{B}
Post-treatment	15.0 ± 1.5^{A}	22.3 ± 5.7^{A}	17.1 ± 2.1^{AB}	17.9 ± 2.1^{AB}	9.3 ± 1.9^{B}	10.6 ± 2.5^{B}
Ψ_{leaf} (MPa)						
Initial	−1.6 ± 0.1^{A}	−1.5 ± 0.2^{A}	−3.2 ± 0.2^{B}	−3.0 ± 0.4^{B}	−4.4 ± 0.2^{C}	−4.6 ± 0.2^{C}
Post-treatment	−1.6 ± 0.1^{A}	−1.6 ± 0.1^{A}	−3.1 ± 0.3^{B}	−2.9 ± 0.4^{B}	−4.2 ± 0.1^{C}	−4.5 ± 0.1^{C}
θ (%)						
Initial	72.9 ± 0.5^{AB}	79.1 ± 2.8^{B}	61.4 ± 1.3^{C}	63.1 ± 2.7^{C}	60.7 ± 0.4^{C}	59.6 ± 2.3^{C}
Post-treatment	86.2 ± 4.1^{A}	80.4 ± 6.3^{A}	78.3 ± 4.1^{AB}	79.3 ± 4.1^{AB}	76.6 ± 6.9^{AB}	71.7 ± 2.4^{B}

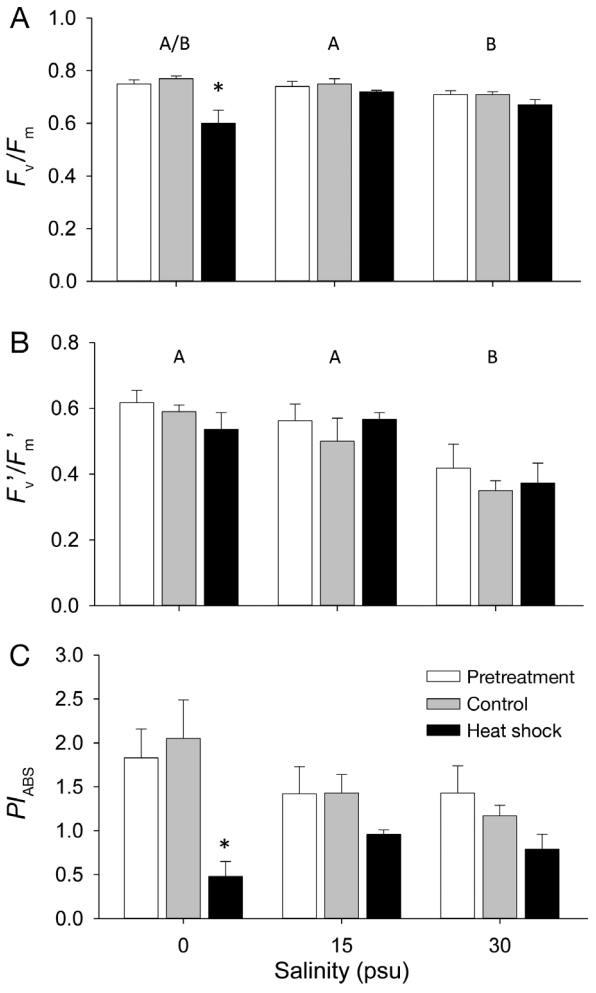

Fig. 1. (A) Potential quantum yield (F_v/F_m), (B) excitation energy capture efficiency of open reaction centers (F_v'/F_m'), and (C) chlorophyll *a* fluorescence performance index (PI_{ABS}) in *Sporobolus pumilus* before and after heat shock. Measurements were collected on plants pre-acclimated to 3 different salinities (0, 15, and 30 psu) for 18 mo. Pretreatment measurements (white bars) were recorded in all plants prior to heat shock. Control plants (gray bars) were exposed to ambient temperatures in darkness, and heat-shocked plants (black bars) were exposed to 45°C for 60 min in darkness. Data are presented as means ± SE. Significant differences associated with salinity are indicated by different letters above treatment groups (3 bars) and differences from pretreatment within each salinity level are indicated by asterisks (n = 4; α = 0.05)

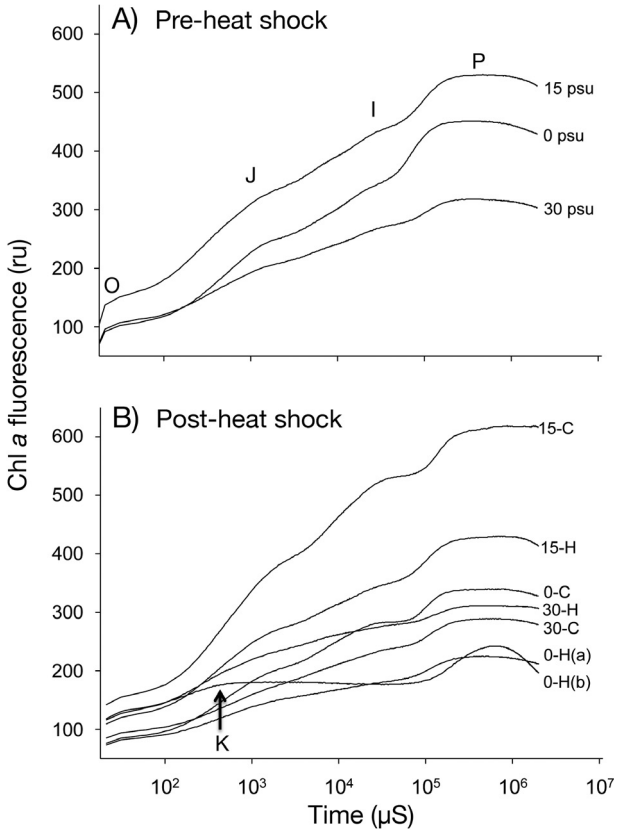

Fig. 2. Typical chlorophyll *a* fluorescence transients (OJIP) for *Sporobolus pumilus* (A) prior to heat treatment and (B) following heat treatment. Salinity treatments (0, 15, and 30 psu) are indicated by numbers following each transient, and letters in the post-heat treatment (Panel B) indicate control (C), heat treatment (H), or 2 different responses observed in heat-treated plants acclimated to freshwater (0-H(a) and 0-H(b)). Note the presence of a K-step (for the 0-H(b) transient; panel B) was observed in approximately 25% of heat-treated plants that were pre-acclimated to freshwater. Values are expressed as relative units (ru)

tively). That is, only *S. pumilus* residing in freshwater had significantly lower PI_{ABS} (compared to pre-treatment and control groups) following heat shock.

Polyphasic chl *a* fluorescence transients (OJIP) reveal distinct phases in fluorescence rise from O to P (Fig. 2). The O-step is the minimum fluorescence when Q_A (primary electron acceptor) is oxidized, and the P-step is the fluorescence when Q_A is fully reduced (Strasser & Srivastava 1995, Kalaji et al. 2014). The intermediate rise, from J to I, is thought to reflect the successive reduction of intersystem electron carriers (i.e. Q_B, plastoquinone, cytochrome, and plastocyanin; Strasser et al. 2004, Yusuf et al. 2010, Chen et al. 2016). During heat stress, typically around 45°C or above, the J- and I-steps can be altered or replaced with a single K-step that typically begins at or around 300 µs (Strasser et al. 2004, Chen et al. 2016). In this study, K-steps with a concomitant loss of J- and I-steps were observed in approximately 25% of the heat-treated *S. pumilus* that were pre-acclimated to freshwater (Fig. 2). Interestingly, no K-step was observed in any heat-treated plants previously acclimated to moderate- or high-salinity conditions. Moreover, there were no significant changes in any polyphasic fluorescence transient

parameters observed between the control and heat-treated *S. pumilus* residing in moderate salinity (15 psu). In contrast, changes in OJIP parameters were apparent in plants exposed to freshwater (0 psu) or high (30 psu) salinity treatments. For these treatments, salinity significantly influenced maximum fluorescence (F_m), whereby lower salinities reported higher values (p = 0.027).

Heat treatments resulted in profound changes in OJIP parameters, especially in plants residing in freshwater (Fig. 3). For example, heat shock resulted in significant increases in the proportion of closed RCs at the J-step (V_j; p = 0.001; Table 3). More specifically, V_j increased by 22 and 11% in 0 and 30 salinity treated plants, respectively. Heat stress also resulted in a significant reduction in Q_A reoxidation rate, as suggested by 83 and 18% increases in M_o for 0 and 30 psu treated plants, respectively (p = 0.001; Table 3). Heat-treated *S. pumilus* residing in freshwater also had a 129% increase in absorption flux per RC (ABS/RC) and a 50% increase in trapping flux per RC (TR_o/RC; p = 0.009 and 0.006, respectively) in comparison to unheated freshwater controls (Fig. 3). Heat-treated plants in freshwater also had a 48% decrease in maximum potential for primary photochemistry (F_v/F_o; p < 0.001), a 47% decrease in elec-

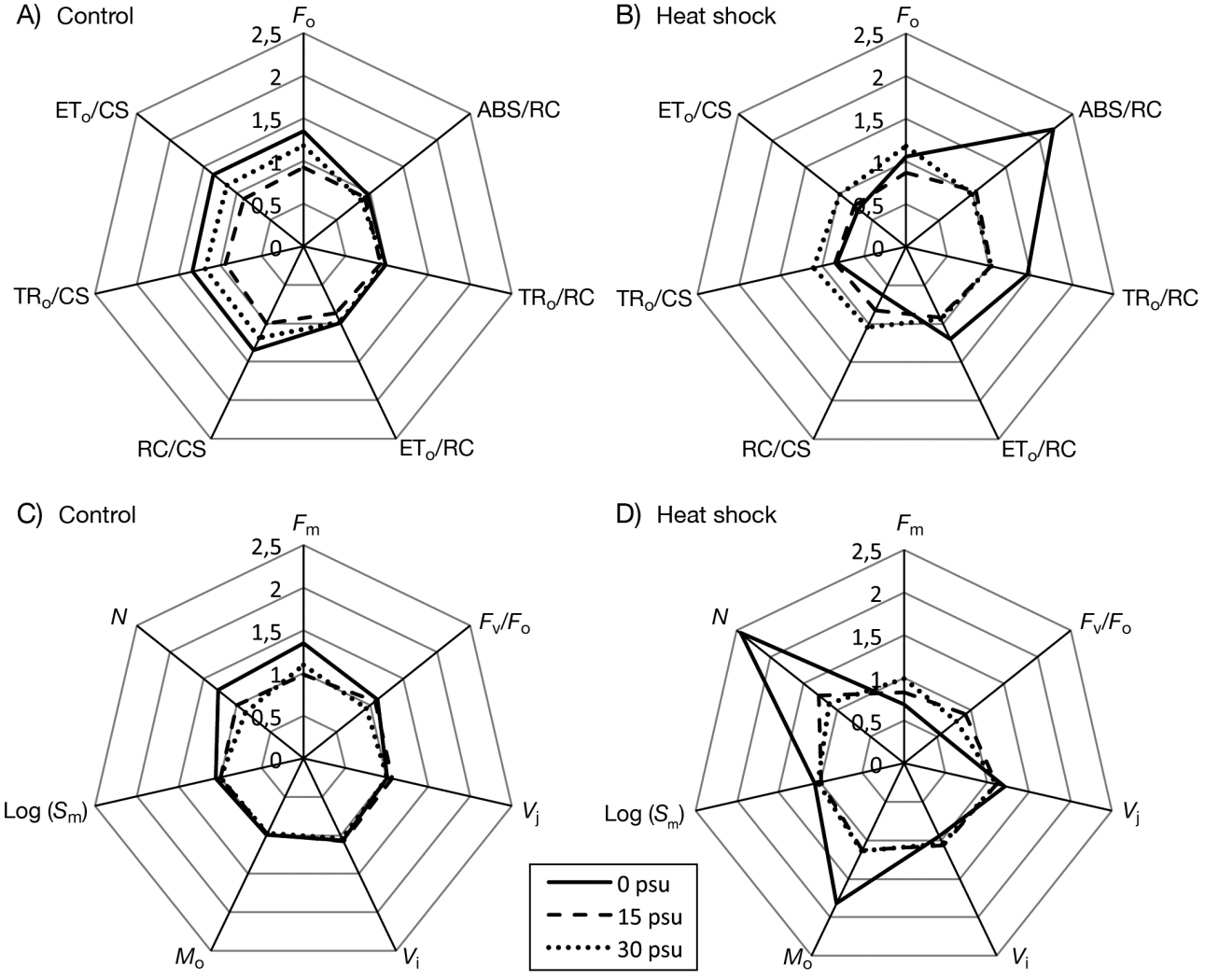

Fig. 3. Spider plots of selective chlorophyll *a* fluorescent induction transient parameters measured in (A,C) control and (B,D) heat-shocked *Sporobolus pumilus*. These parameters help characterize the behavior of Photosystem II following heat shock in plants pre-acclimated to 3 different salinities: 0 (solid lines), 15 (dashed lines), and 30 psu (dotted lines). Parameters were calculated from fluorescent transients, wherein the presented values were normalized as a relative proportion of the initial (pre-heat shock) measurements (e.g. a doubling of a particular response would have a value of 2). Definitions of fluorescent parameters used in these plots can be found in Table 1

Table 3. Chlorophyll *a* fluorescent output parameters recorded following heat-shock treatments on *Sporobolus pumilus* acclimated to different salinities (0, 15, and 30 psu). Statistical differences between control and heat treatments within each salinity class are indicated by asterisks. Data are presented as means ± 1 SE (n = 4; α = 0.05). Definitions and mathematical expressions of each fluorescent output parameter reported can be found in Table 1

Parameter	0 psu-Control	0 psu-Heat	15 psu-Control	15 psu-Heat	30 psu-Control	30 psu-Heat
F_o	118.5 ± 7.5	92.7 ± 10.5	119.3 ± 6.4	112.0 ± 7.9	103.5 ± 13.1	103.8 ± 7.9
F_m	528 ± 27	268 ± 42*	507 ± 47	427 ± 30	364 ± 51	328 ± 35
F_v/F_o	3.5 ± 0.32	1.8 ± 0.32*	3.2 ± 0.30	2.7 ± 0.08	2.5 ± 0.17	2.1 ± 0.24
V_j	0.46 ± 0.01	0.56 ± 0.02*	0.51 ± 0.01	0.52 ± 0.01	0.46 ± 0.006	0.51± 0.02*
V_i	0.78 ± 0.01	0.69 ± 0.06	0.81 ± 0.01	0.79 ± 0.01	0.76 ± 0.01	0.76 ± 0.02
M_o	0.78 ± 0.06	1.43 ± 0.26*	0.89 ± 0.03	1.02 ± 0.03	0.85 ± 0.01	1.00 ± 0.06*
Log S_m	2.76 ± 0.01	2.72 ± 0.11	2.75 ± 0.09	2.84± 0.03	2.73 ± 0.04	2.83 ± 0.03
N	892 ± 67	1706 ± 782	1074 ± 233	1379 ± 135	1044 ± 99	1345 ± 99
ABS/RC	2.16 ± 0.15	4.95 ± 1.29*	2.34 ± 0.14	2.67 ± 0.09	2.54 ± 0.04	2.90 ± 0.14
TR_o/RC	1.66 ± 0.07	2.49 ± 0.38*	1.75 ± 0.04	1.94 ± 0.05	1.81 ± 0.01	1.92 ± 0.04
ET_o/RC	0.88 ± 0.02	1.06 ± 0.13	0.86 ± 0.03	0.91 ± 0.04	0.96 ± 0.01	0.92 ± 0.02
ET_o/CS	48.5 ± 2.0	25.5 ± 5.2*	44.8 ± 4.3	38.5 ± 2.4	39.2 ± 5.0	33.5 ± 3.0
RC/CS	54.9 ± 2.9	25.5 ± 7.0*	51.2 ± 3.8	41.9 ± 2.1	40.8 ± 5.6	35.9 ± 2.8
TR_o/CS	91.4 ± 4.7	56.9 ± 9.2	90.2 ± 6.3	81.7 ± 5.7	74.0 ± 9.7	69.5 ± 5.8

tron transport flux per excited cross section (ET_o/CS; $p < 0.001$), and a 54% decrease in the density of Q_A reducing PSII RCs (RC/CS; $p < 0.001$; Fig. 3). It is important to note that changes in ABS/RC, TR_o/RC, F_v/F_o, ET_o/CS, and RC/CS were not observed in heat-treated *S. pumilus* receiving salinity treatments (i.e. 15 and 30 psu; Table 3).

4. DISCUSSION

As expected, salinity treatments promoted notable changes in plant–water relations for *Sporobolus pumilus*. Specifically, there were declines in g, Ψ_{leaf}, and θ as salinity increased from 0 to 30 psu. Similar salinity-associated responses have been reported in other studies (Khan et al. 2000, Salpeter et al. 2012), and were attributed to lower soil-water potentials (Ψ_{soil}) fostered by high solute levels within interstitial pore-waters (Salpeter et al. 2012). That is, plants must maintain a gradient of decreasing water potentials to achieve water flux through the soil–plant–atmosphere continuum. Water potential (Ψ) of pure water is designated at 0 MPa, and any addition of salts or other solutes will effectively lower Ψ. In the absence of any physiological modifications, an increase in soil solutes would lower Ψ_{soil} and osmotically withhold water from plant roots (Larcher 2003, Touchette et al. 2009b, Salpeter et al. 2012). Therefore, to promote water influx and positive turgor pressure, plants may lower Ψ within developing and/or existing tissue by increasing internal solute concentrations (Pezeshki & DeLaune 1993, Flowers & Colmer 2008, Hessini et al. 2008). In *S. pumilus*, these physiological adjustments following sudden increases in salinity (including lower g, Ψ_{leaf}, and θ) may take several weeks to fully develop (Salpeter et al. 2012). Therefore, extending pre-acclimation in some halophytes may be necessary to capture long-term physiological modifications to higher salinities.

In some areas, coastal marsh systems must contend with unusually high summer temperatures. In *Salicornia–Distichlis* marshes, for example, sediment surface temperatures can reach 45°C (Teal 1958). Similarly, surface temperature measurements using *Littoraria irrorata* biomimic sensors attached to *Sporobolus alterniflorus* (syn. *Spartina alterniflora*) stalks revealed temperatures exceeding 45°C (Iacarella & Helmuth 2011). High temperatures can adversely affect a number of cellular and metabolic processes in plants (e.g. loss of membrane stability, enzyme inactivation, inhibition of protein synthesis; Wahid et al. 2007). Photosynthesis is particularly vulnerable to elevated temperature stress, as high temperatures can promote a range of physiological perturbations such as inhibition within the oxygen-evolving complex, disruption of electron transport reactions, and diminished PSII photochemical efficiencies (Chen et al. 2016, Stirbet et al. 2018). Potential quantum yield (F_v/F_m) is widely used as an indicator of PSII function and reflects the trapping efficiency of absorbed light to reduce the primary electron acceptor, Q_A (Li et al. 2009). Often, declining F_v/F_m values represent some form of PSII damage or photoinhibition during environmental stress (Baker 2008, Ogaya et al. 2011). In this study, high salinity (30 psu) resulted in a small,

but significant, decline in F_v/F_m for *S. pumilus* (when compared to non-heat-treated plants residing in freshwater and moderate salinities). This response is consistent with other studies wherein elevated salinities fostered F_v/F_m declines in photosynthetic tissues, including the macroalga *Ulva lactuca*, and coastal graminoids *Phragmites australis* and *Juncus roemerianus* (Xia et al. 2004, Deng et al. 2011, Touchette et al. 2012). Interestingly, heat shock also resulted in a significant F_v/F_m decrease in this study, but this phenomenon was only observed in *S. pumilus* acclimated to freshwater. In contrast, the excitation capture efficiency of open PSII RCs, F_v'/F_m', was not affected by heat treatments.

Some studies have shown that F_v/F_m is not always sensitive to heat stress, and others have expressed concern that this parameter lacks specific details regarding alterations within PSII behavior (Strasser et al. 2000, Crafts-Brandner & Salvucci 2002, Wen et al. 2005). The fluorescent marker PI_{ABS} offers a broader evaluation of PSII performance by considering processes related to active RC densities, primary photochemistry, and electron transport (Strasser et al. 2000, Stirbet et al. 2018). PI_{ABS}, therefore, is responsive to changes in antenna properties, trapping efficiencies, and electron transport beyond Q_A (Kalaji et al. 2014). In this study, there was no salinity-induced response for PI_{ABS}, although high variability within this parameter may, in part, contribute to a lack of statistical difference. Nevertheless, PI_{ABS} did decline in heat-treated plants that were previously acclimated to freshwater. Heat-stress-induced declines in PI_{ABS}, as with F_v/F_m, were not observed in salinity-acclimated plants, suggesting that *S. pumilus* pre-acclimated to environmental salts may have greater PSII thermostability compared to plants residing in strictly freshwater. Similar physiological responses, where exogenously supplied salts (e.g. NaCl or $CaCl_2$) promoted PSII thermostability, were observed in other species including *Artemisia anethifolia*, *Nicotiana tabacum*, *Sorghum bicolor*, and *Suaeda salsa* (Lu & Zhang 1998, Lu et al. 2003, Wen et al. 2005, Tan et al. 2011, Yan et al. 2012). Moreover, studies have shown that low osmotic potentials (often attributed to low water stress) within leaf tissues are also correlated with PSII stability during heat stress (Seemann et al. 1986, Havaux 1992). Yan et al. (2012) suggested that the accumulation of osmoprotectant(s) (e.g. glycinebetaine or proline), could possibly contribute to enhanced thermoresistance in plants exposed to osmotic stressors (salt or drought), as these compounds tend to stabilize subcellular structures, control free radical accumulation, and buffer cellular redox potentials (Lu et al. 2003, Ashraf & Foolad 2007).

Closer inspection of polyphasic fast chl *a* fluorescent kinetics, as derived from OJIP transients, can provide some insight into possible physiological mechanisms that promote PI_{ABS} and F_v/F_m declines in heat-treated plants grown in freshwater. The initial slope (M_o) and the variable fluorescence at the J-step (V_j) are useful in calculating the trapping flux (TR) per active PSII RC ($TR_o/RC = M_o/V_j$; Stirbet et al. 2018). Using these metrics, TR_o/RC reflects the maximal rate of RC closures across all RCs that can functionally close (Wen et al. 2005). During stress conditions, it is possible that some RCs no longer close and, instead, serve as heat sinks or 'silent RCs' that do not reduce Q_A to Q_A^- (Strasser et al. 2004). Thus, TR_o/RC only refers to RCs that are photochemically active and can reduce Q_A. In this study, increases in TR_o/RC (observed in heat-treated freshwater plants), likely indicate inactivation of PSII RCs and/or a decrease in re-oxidation rates of Q_A (Wen et al. 2005). Inactivation of RCs, however, is further supported by lower active PSII RC densities (i.e. lower RC/CS) observed in heat-treated plants acclimated to freshwater. Interestingly, the number of active RCs was considerably lower in heat-treated plants acclimated to freshwater only, suggesting that salt exposure for plants in the other treatments offers some level of thermoprotection of PSII with improved RC thermostability. Chl *a* fluorescent transients in heat-treated freshwater plants also revealed a K-step in approximately 25% of the plants. The K-step is well documented in heat-stress studies involving plants, and is thought to be attributable to disruptions in the donor side of PSII, especially the destruction of the oxygen-evolving complex through a dissociation of the manganese cluster (Chen et al. 2016, Stirbet et al. 2018). The lack of an observed K-step in salt-acclimated plants following heat-shock also demonstrates enhanced thermotolerance of PSII, and suggests that exposure to environmental salts may also improve the thermostability of the oxygen-evolving complex (Mathur et al. 2013).

Plant physiological behavior associated with simultaneous multiple stressors can be difficult to predict due to the often complex nature of their responses. Such responses may either be reinforcing or offsetting (Aber et al. 2001). In this study, the presence of salt promoted some degree of salt stress in *S. pumilus* as indicated by changes in plant–water relations and quantum yield. Similarly, high-temperature shock alone also promoted plant stress as indicated by lower PI_{ABS}, F_m, and F_v/F_o, and higher V_j, M_o, and TR_o/RC.

While it may seem intuitive that the combined stressors, involving disruptions in both plant–water relations and PSII, would foster additive responses compromising the overall ability of a plant to survive, this was not observed in *S. pumilus*. Indeed, the presence of environmental salts appeared to offset the expected heat-related responses, allowing these halophytes to seemingly tolerate unusually high temperatures. More specifically, it would appear that saline porewaters increased the resistance of PSII to heat stress by minimizing initial disturbances within the PSII RCs and subsequent disruptions within the oxygen-evolving complex. Similar responses have been observed in the halophyte *S. salsa* found in northern China, where it is adapted to high-saline soils with summer temperatures approaching 45°C (Lu et al. 2003). While specific mechanisms that promote greater thermotolerance in plants exposed to environmental salts are unclear, it is possible that compatible solute accumulations (e.g. betaines, proline, and sugar alcohols) in plants experiencing osmotic stress (e.g. saline systems or drought) may foster some protective benefits to PSII processes. Glycinebetaine, for example, has been shown to stabilize the oxygen-evolving complex and protect the PSII core from heat stress (Papageorgiou & Murata 1995, Allakhverdiev et al. 2003, Yang et al. 2007). This compound has also been shown to accumulate in chloroplasts during periods of osmotic stress (Robinson & Jones 1986). Regardless of the physiological mechanisms involved in promoting thermotolerance in *S. pumilus*, such responses would likely play a role in species occurrence and distribution, especially as global temperatures rise (Touchette et al. 2019). That is, *S. pumilus* also occurs in coastal freshwater systems (e.g. dune swales) that lack any direct exposure to seawater. It is conceivable that these freshwater populations will be more vulnerable to rising global temperatures relative to their counterparts residing in saline-rich salt marshes.

Acknowledgements. Support for this study was generously provided by the North Carolina Sea Grant (NCSG), UNC Water Resources Research Institute (WRRI), US Geological Society, and Elon University Department of Biological Science. We thank 2 anonymous reviewers for their comments and insights that helped strengthen the final manuscript.

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Editorial responsibility: Anna Metaxas, Halifax, Nova Scotia, Canada

Submitted: January 10, 2020; Accepted: June 19, 2020
Proofs received from author(s): August 21, 2020