Effects of Salinity and Temperature on Respiratory Metabolism of *Salicornia utahensis* From a Great Basin Playa

Lyneen C. Harris
M. Ajmal Khan
Jiping Zou
Bruce N. Smith
Lee D. Hansen

Abstract—Plants that live in the desert playas of the Great Basin must simultaneously tolerate very high concentrations of salt and high temperature. This study characterizes the respiratory metabolism of one species growing in this environment. An isothermal calorimetric method was used to measure the dark metabolic heat rate (q) and CO_2 production rate (R_{CO2}) of stem tissue from *Salicornia utahensis* grown in the greenhouse or in constant temperature growth chambers at six different concentrations of NaCl solutions ranging from 0 to 1.0 M. Metabolic rates were measured at eight temperatures from 5 to 40 °C. Temperature and salt dependencies of q and R_{CO2} were used to model response of both growth and substrate carbon conversion efficiency. Salt and temperature stresses are not additive, but rather oppose one another, in other words, the higher the salt, the higher the temperature the plants will tolerate and vice versa. The maximum temperature tolerated is an approximately linear function of salt concentration, being about 20 °C at 0 M salt and about 32 °C at 1 M. Concentration of the endogenous osmoticant glycinebetaine increased with salt and temperature but only to about 20 °C and 900 mM NaCl.

There are many indicators of stress responses of plants such as chlorophyll fluorescence, heat shock proteins, and osmoticant molecules. Each of these tells something about the way a plant responds to a stress. Ultimately in order to understand plant survival and growth, we must know how the particular stress affects the energy metabolism (Smith and others 2000). This study measured the effects of salt (NaCl) and temperature on the biomass accumulation and respiratory metabolism of a desert halophyte, *Salicornia utahensis* Tidestr. This species is adapted to growth and survival in wet, salty soils and a cold desert climate.

Growth of halophytes is stimulated by some level of salinity (Flowers and others 1986), however growth of most species is inhibited by too high salt concentrations, in other words, greater than about 0.5 M NaCl, the concentration of salt in seawater (Ungar 1991). Previous studies on growth of desert species (*Atriplex griffithii, Halopyrum mucronatum, Haloxylon recurvum,* and *Sueda fruticosa*) from Pakistan showed that low salinities promoted growth (Khan and others 1998). Increasing salt to 425 mM promoted growth of *Cressa cretica* but growth in 850 mM salt was not significantly different from controls grown without added salt (Khan and Aziz 1998). Growth of halophytes from the Great Basin in Western North America (*Salicornia rubra, Salicornia utahensis, Suaeda torreyana, Allenrolfea occidentalis*) shows a similar pattern of promotion at moderate salinities (400 to 600 mM NaCl) and a decline with further increases in salinity (Khan and others 2000).

Halophytes usually absorb a considerable amount of salt to maintain osmotic balance with their highly saline medium. The most salt tolerant species have high internal salt concentrations, suggesting that the ability of the cells to tolerate high salt concentrations is at least as important to survival as the ability to restrict accumulation of salt (Flowers and others 1977). However, the enzymes of halophytes, unlike those of salt tolerant bacteria, are inhibited by high salt concentrations suggesting that cytoplasmic enzymes are protected from salt either by sequestration of salt in cell walls and vacuoles (Weber and others 1977) or by protective solutes such as glycinebetaine, proline, polyols, or cyclitols (Flowers and others 1977).

Seed germination of *Triglochin maritima* from the Great Basin was most inhibited by exposure to high salinities at suboptimal thermoperiods (Khan and Ungar 1999). For halophytes *Salicornia rubra, S. utahensis, Distichlis spicata,* and *Allenrolfea occidentalis* growing in the field showed the highest metabolism, respiration, efficiency, and growth during May and June and lowest during the hot, dry month of August (Harris and others, in press). By contrast, *Salicornia europaea* from a salt marsh in Ohio germinated better following rain events in June than earlier in the year (Egan and Ungar 1999).

Except for the fact that *S. utahensis* is a common plant in the Great Basin desert with cold winters, hot summers, and large diurnal temperature changes (commonly 20 to 30 °C), little is known about the temperature tolerance of this species. During the period of high summer temperatures, which is also the time of least rainfall, water evaporates from the shallow playas, increasing salt concentration in the soil. It was thus of interest to examine the combined effects of temperature and salt. In their saline environment,

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Lyneen C. Harris is an Undergraduate Student; M. Ajmal Khan is a Visiting Professor; Jiping Zou is a Research Associate; Bruce N. Smith is a Professor, Department of Botany and Range Science; Lee D. Hansen is a Professor, Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602.

halophytes are normally exposed to multiple stresses. In addition to salt and temperature stresses, there is often a low osmotic potential of water and a low partial pressure of oxygen due to standing water or a high water table (Ungar 1991). Presumably the imposition of one kind of stress reduces tolerance to other simultaneous stresses, a hypothesis looked at in this study. This study examines the interaction of both low and high salt and temperature stresses. Predicted biomass accumulation and characteristics of energy metabolism are used as indicators of stress response.

Aerobic respiration has two aspects: catabolism and anabolism. In catabolism, organic substrates are oxidized to produce CO_2. Part of the energy produced by oxidation is used to convert ADP and inorganic phosphate to ATP, the rest is lost as heat.

$$\text{substrate} + O_2 + \text{ADP} + P_i \rightarrow CO_2 + H_2O + \text{ATP} + \text{heat} \quad (1)$$

ATP produced in catabolism is transient, but is used for cellular work, including anabolism as shown below:

$$\text{substrate} + \text{ATP} \rightarrow \text{growth} + \text{ADP} + P_i + \text{heat} \quad (2)$$

In anabolism, heat and new plant tissue are produced and ATP is hydrolyzed back to ADP and phosphate. A calorimeter measures the rate of heat loss (q) from both catabolism and anabolism. The rate of CO_2 production (R_{CO2}) measures the rate of catabolism. With carbohydrate as the specific substrate, predicted growth rate of structural biomass or rate of anabolism (R_{SG}) is thus related to the difference in two measured variables as in equation 3,

$$R_{SG}\Delta H_B = 455R_{CO2} - q \quad (3)$$

where ΔH_B is the enthalpy change for the formation of biomass from photosynthate and Thornton's constant (–455 ± 15 kJ mol^{-1} of O_2) is incorporated to calculate the rate of energy generated by catabolism. Thus, growth rate in terms of energy is proportional to the difference between the measured values of R_{CO2} and q. The temperature dependencies of R_{CO2} and q are different (Hansen and others 1994). The difference between $455R_{CO2}$ and q therefore changes with temperature and this difference can be used to predict growth rate changes with temperature (Criddle and others 1997).

Predicted specific growth rate may also be expressed as a function of the substrate carbon conversion efficiency (ε) and respiration rate (R_{CO2}).

$$R_{SG} = R_{CO2}[\varepsilon/(1-\varepsilon)] \quad (4)$$

Combining equations 3 and 4 to eliminate R_{SG} gives equation 5,

$$[\varepsilon/(1-\varepsilon)]\Delta H_B = -q/R_{CO2} - (1-\gamma_P/4)455 \quad (5)$$

which relates the ratio of q/R_{CO2} to ε. Values of q/R_{CO2} measured as a function of temperature can thus provide information on substrate carbon conversion efficiency (ε) and the oxidation state of the substrate carbon, in other words, γ_P (Hansen and others 1994).

The purpose of this work is to examine how an extreme halophyte adapts its respiratory metabolism to temperature and salinity conditions common in its native habitat. In this study, calorimetry was used to determine the high and low stress temperatures and salinities for *Salicornia utahensis* grown in controlled conditions. When the metabolic heat rate exceeds energy made available through catabolism of carbohydrate, the plant is considered to be stressed (Smith and others 2000).

Materials and Methods

Seeds of *Salicornia utahensis* Tidestr. were collected during the fall of 1995 from salt playas 1–2 km east of Goshen, Utah. Seeds were separated from the inflorescence, surface sterilized using the fungicide Captan, and stored at 4 °C. Seeds were germinated in 36 cm^2 pots filled with sand and placed in trays of distilled water to sprout. Once sprouted, salt was introduced into the trays and increased by 200 mM every 2 days until desired salinity was reached. Ten pots each were grown at 0, 200, 400, 600, 800, and 1,000 mM NaCl. A half-strength Hoagland solution was used to supply nutrients. Pots were subirrigated and the water level adjusted daily to correct for evaporation. Salt solutions were completely replaced once a week to avoid buildup of salinity in the pots. Plants were grown in a greenhouse at a daytime mean temperature of 25 °C and a mean nighttime temperature of 15 °C. Metabolic heat rates (q) and respiration rates (R_{CO2}) and fresh and dry weights of plant shoots were measured 60 days after the highest salt concentration was reached.

Another set of plants was sprouted as above but grown at six different salinities in each of four controlled environmental chambers maintained at 10, 20, and 30 °C with a 12-hour photoperiod (200 μmol m^{-2} s^{-1}, 400–700 nm). Plants were harvested after about 5 months.

Plants were cut just below the cotyledon and about 100 mg fresh wt. of tissue was placed in each of three ampules of a microcalorimeter (Hart Scientific model 7707 or Calorimetry Sciences Corporation model 4100). After 15 to 20 minutes thermal equilibration at the desired temperature, the metabolic heat rate (q) was measured for another 15 to 20 minutes. The ampules were removed from the calorimeter and a small vial filled with 40 μl of 0.4 M NaOH placed in the calorimeter ampule with the tissue. Again a 15–20 minute thermal equilibration was necessary, followed by measurement of the sum of the heat rate from metabolism and CO_2 reaction with the NaOH (–108.5 kJ $mole^{-1}$) for 15–20 minutes. After the NaOH was removed, the heat rate (q) is measured again as before (Hansen and others 1994; Criddle and others 1997). The difference in the measurements with and without NaOH solution gives the rate of CO_2 evolution (R_{CO2}) by the plant tissue. The tissue was then run at another temperature. Measurements were made on each sample at 9 temperatures: 5, 10, 15, 20, 25, 30, 35, 40, and 45 °C.

For glycinebetaine measurements, the method of Gorham (1984) was followed. Plant material (0.5 g) was boiled in 10 ml of water for 2 hours at 100 °C in a dry heat bath. Samples were diluted with a 50 mM potassium dihydrogen phosphate buffer adjusted to pH 4.6. This was the carrier buffer that was also used in the HPLC system. The sample was cooled and filtered using a 0.45 μm membrane filter (Gelman, Ann Arbor, MI), and then used directly to measure glycinebetaine with a Hewlett Packard 1050 modular 3D HPLC (Boise, ID) with quaternary pump, online degasser, autosampler, and a diode array detector with a stainless steel flow cell (6-mm path length, 8 μl volume). Separations were performed on a 250 x 4 mm i.d. stainless steel column

Table 1—Endogenous levels of glycinebetaine in seedlings of *Salicornia utahensis* grown in different NaCl concentrations in the greenhouse.

NaCl concentrations, mM	g glycinebetaine/kg dry wt.
0	28.5
300	48.8
600	45.6
900	44.4
1,200	37.0
1,500	47.6

Table 2—Endogenous levels of glycinebetaine in seedlings of *Salicornia utahensis* grown at different temperatures in growth chambers in different NaCl concentrations.

Growth temperature	Salinity mM NaCl	Glycinebetaine g/kg dry weight
10 °C	0	34.4
	300	32.3
	900	46.3
	1,500	35.7
20 °C	0	14.9
	300	57.1
	900	59.4
	1,500	37.7
30 °C	0	ND
	300	54.2
	900	died
	1,500	died

packed with 10 μm Nucleoside 100-10SA (Phenomenex, Torrance, CA). Flow rate was 1.2 ml min^{-1}. Glycinebetaine standards were run at 1, 10, and 100 mmol l^{-1}.

Results and Discussion

Glycinebetaine concentrations (table 1) increased from 28.5 g glycinebetaine per kg dry wt. of plant tissue for *Salicornia utahensis* grown in zero salt to 48.8 g/kg for plants grown in the greenhouse in 300 mM NaCl. Increases in salinity above 300 mM had essentially no effect on the glycinebetaine concentration within the plant tissue. Temperatures at which the plants were grown (table 2) did affect glycinebetaine concentration. Compared with plants grown in zero salt at 20 °C, plants grown without salt at 10 °C had more than twice the concentration of glycinebetaine. Perhaps the glycinebetaine was not functioning as an osmoticant in this case. Unlike some reports in the literature (Khan and others 1998), there was not a linear increase in glycinebetaine with salt exposure. Other osmoticants exist (Flowers and others 1977) and may be operative as well here. Weber and others (1977) did show that salt tolerance in *Salicornia utahensis* was based on exclusion of salt from the photosynthetic cells and on the ability of the succulent stem to function even though sections were dead owing to high salt concentration. It may be that glycinebetaine operates at relatively low concentrations of NaCl but that active accumulation of salt into certain tissues occurs with higher concentrations of external salt and possibly at higher temperatures.

Figure 1 is a plot of the metabolic heat rate as a function of salt concentration at the various temperatures. The data show the heat rate generally increases with increasing

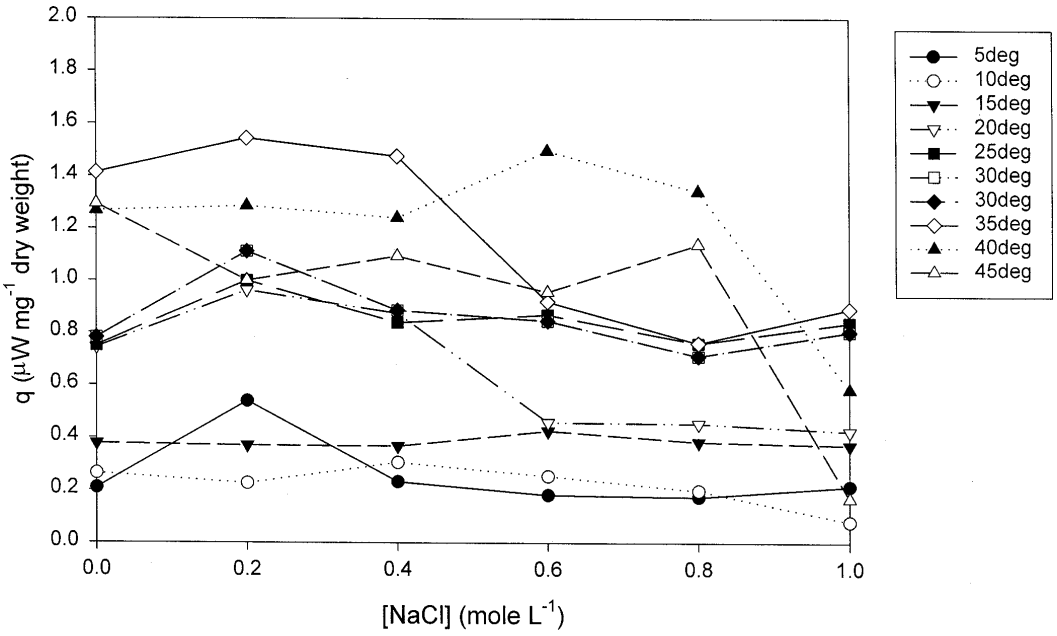

Figure 1—Plot of dark metabolic heat rate q versus the NaCl concentration in which the plants were grown. Each line presents the measurements made at the indicated temperature.

temperature up to 35 to 40 °C after which it decreases markedly. Metabolic heat rate generally decreases with increasing salt, the effect becoming more pronounced with increasing temperature.

Figure 2 is a plot of the respiratory CO_2 rate (R_{CO2}) as a function of salt concentration at the various temperatures. R_{CO2} increases with temperature at low temperatures, goes through a maximum at intermediate temperatures, then decreases at higher temperatures. Salt has little effect on R_{CO2} except at very low (≤5 °C) and very high (>35 °C) temperatures where R_{CO2} increases sharply.

Predicted growth of *S. utahensis* seedlings generally increased with salt concentrations (fig. 3), with high temperatures showing the largest effect of increasing salt.

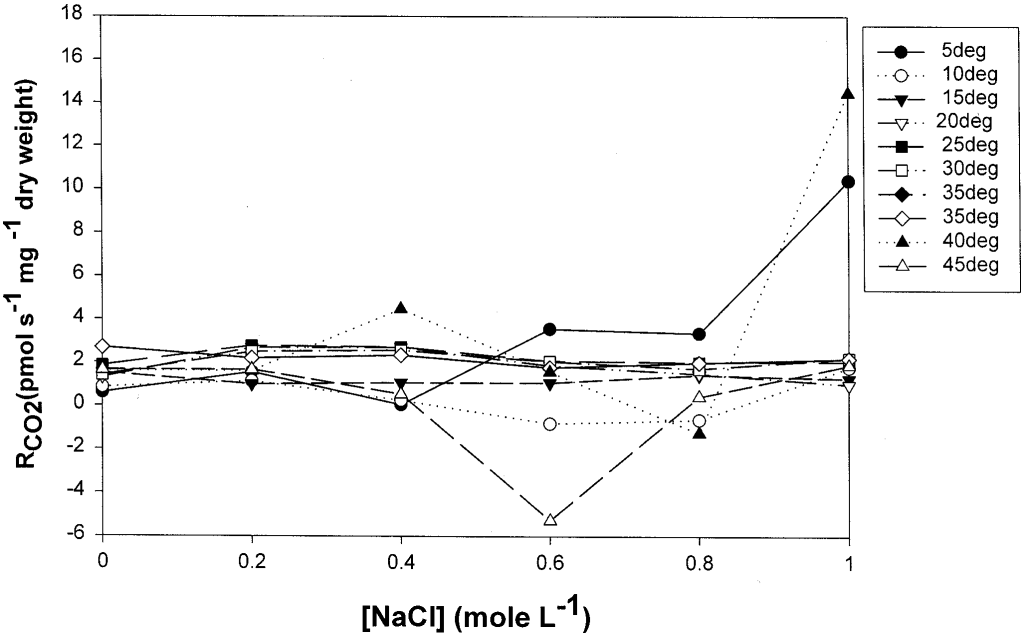

Figure 2—Plot of dark metabolic CO_2 rate versus the NaCl concentration in which the plants were grown. Each line presents the measurements made at the indicated temperature.

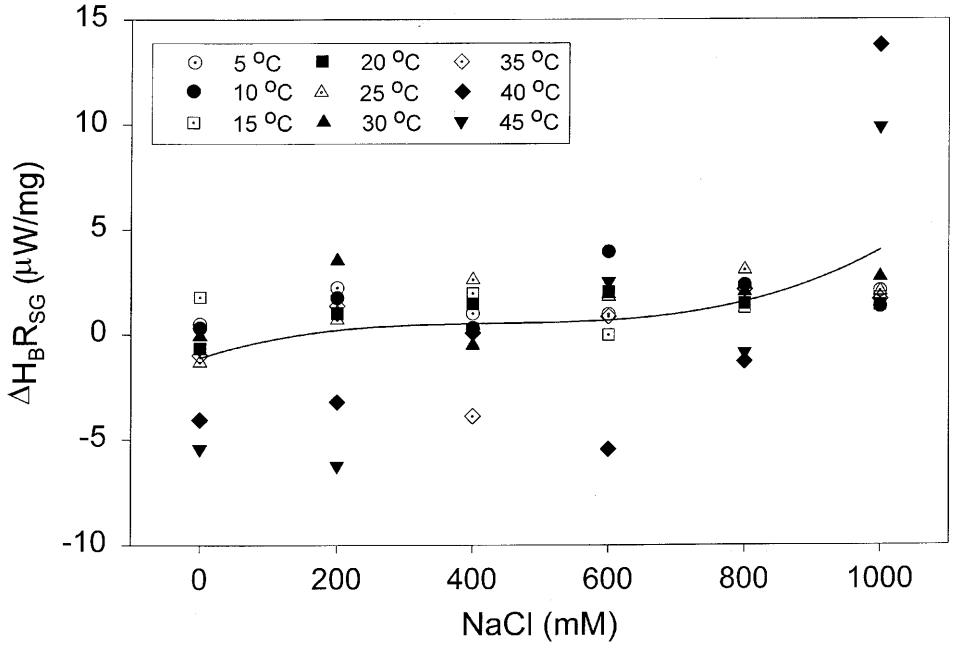

Figure 3—Specific growth rate, $\Delta H_B R_{SG}$, calculated from metabolic heat and CO_2 rates as a function of the salt concentration in the growth medium.

Plotting the same data another way (fig. 4), averaging the growth for all salt concentrations at each temperature predicts a decrease in growth with increasing temperature. The two data points at 1,000 mM NaCl at 40 and 45 °C are anomalous in both figures 3 and 4. These two points at high values of $\Delta H_B R_{SG}$ do not indicate high growth rates, instead these values are a result of the imposed stress and indicate the production of catabolic products other than CO_2.

Figure 5 is a contour plot of the specific growth rate, $\Delta H_B R_{SG}$, calculated from metabolic heat and CO_2 rates as a function of the measurement temperature and salt concentration in the growth medium. The synergism between these

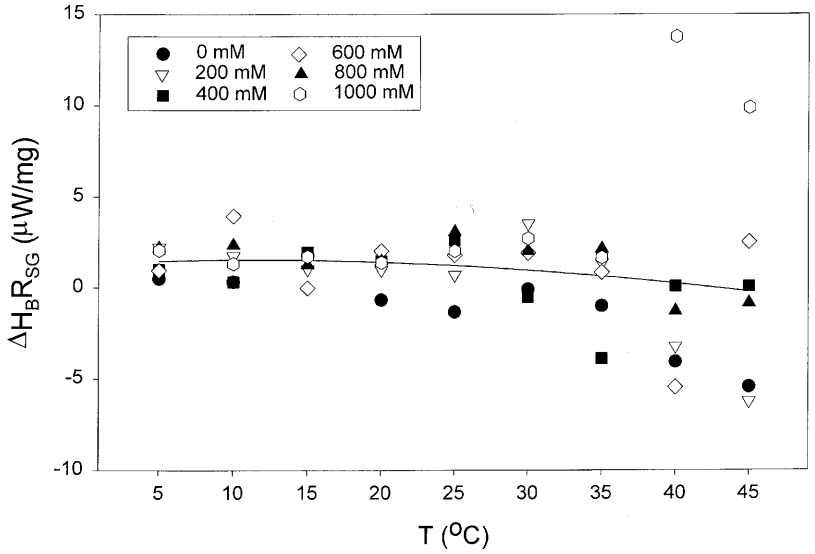

Figure 4—Specific growth rate, $\Delta H_B R_{SG}$, calculated from metabolic heat and CO_2 rates as a function of the measurement temperature.

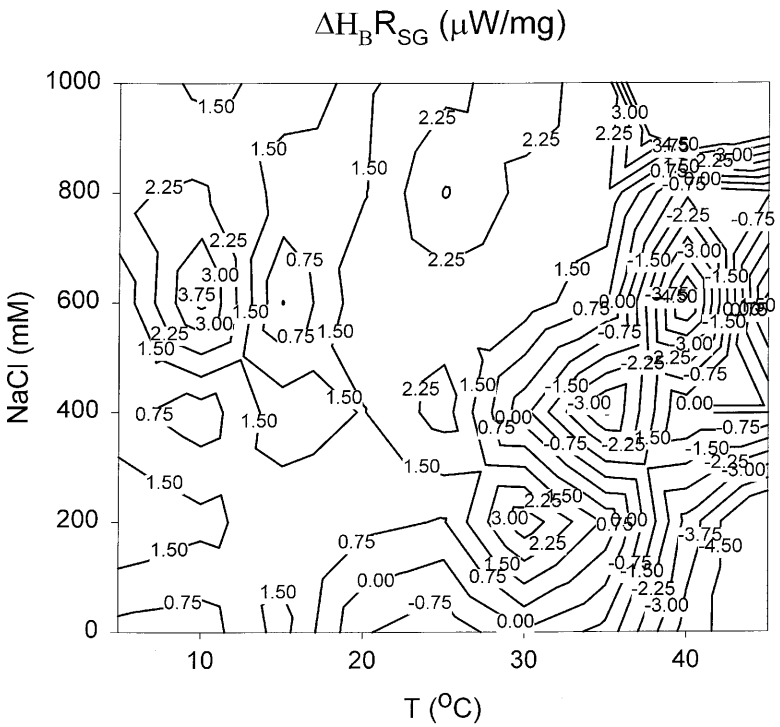

Figure 5—Contour plot of the specific growth rate, $\Delta H_B R_{SG}$, calculated from metabolic heat and CO_2 rates as a function of the measurement temperature and salt concentration in the growth medium.

two environmental variables can be deduced from this plot. Contrary to our intuitive assumption that high temperature and high salt would be more stressful than either stress alone, the results show that the two together are less stressful than either high salt or high temperature alone. This is shown by the approximately linear diagonal of maximum tolerable temperatures that runs from about 20 °C at 0 salt to about 30 °C at 1,000 mM salt on the contour plot. Note that $R_{SG}\Delta H_B$ tends toward negative values to the right of this diagonal.

The major conclusion of this paper agrees with the natural history of *S. utahensis*. In the Great Basin, precipitation occurs primarily during early spring (April-May) and late fall (October-November) when temperatures are typically around 5 °C at night and 20 to 25 °C during the day. Thus, the salt in the playas is most dilute during periods with these temperatures. In the summer, when daytime air temperatures range up to about 45 °C, is also when the salt is most concentrated. The data collected in this study show that *Salicornia utahensis* respiratory metabolism is well adapted to these conditions, that is, to lower salt concentrations at low temperatures and higher salt concentrations at higher temperatures. The details of how the species has accomplished the feat of adapting to the sum of two apparently deleterious stresses are certainly not clear as yet.

Halophytes are normally "includers" (in other words, they are generally in equilibrium with the salt in solution in the root environment), and thus there is probably little or no extra energy cost for operating in a variable, high-salt environment. Tertiary structures of proteins and membranes must however be appropriate for the environment.

In summary, the methods employed in this study have contributed to the understanding of the phenomenon of salt stress by providing information on the synergism between salt and temperature.

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