THE GROWTH AND WATER RELATIONS OF A
COASTAL HALOPHYTE, SALICORNIA BIGELOVII

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Jon Randall Weeks entitled The Growth and Water Relations of a Coastal Halophyte, Salicornia bigelovii and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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This research is not what it started out to be. It began as a simple greenhouse experiment intended to reveal the relationship between growth and salinity in a cosmopolitan euhalophyte. At this juncture it encompasses work in the greenhouse, laboratory and estuary and requires integrated conceptualizing at the biochemical, cellular, whole plant and ecological levels. Furthermore, the project is much larger now than when it began. I believe that's a good sign.

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ABSTRACT

The succulent, annual euhalophyte, *Salicornia bigelovii* was grown in 1, 10, 35, 45 and 60 ppt Instant Ocean. This range represents approximately 1/35 to nearly twice the salinity of seawater. The plants in the 4 highest salinities had common final dry weights and seed yields of about 60 and 11 g, respectively, while the 1 ppt plants had 28 and nearly 5 g, respectively. The water relations data reflected the growth and seed production of the plants. The plants in the 4 higher salinities had water potentials sufficient to generate large import gradients and osmotic potentials which contributed to substantial turgors. The 1 ppt plants had a gradient like the rest, but a very low turgor of 0.11 MPa which was barely 23% of that of the lowest of the other treatments. Higher salinities resulted in slightly greater organic and inorganic osmotica contents. Overall, these results suggest a relatively fixed genetic response to a wide range of salinities, as well as an inability to function well at very low salinities. No plant grown at 0 ppt was ever able to reproduce. Therefore, this plant is an obligate halophyte.

Experiments in the plant's native coastal estuary indicated meristem water potentials fluctuate with the tides, although they remain about 1.5 MPa below the corresponding soil water potentials. The plants occupy a discrete elevational range throughout the estuary, spending about 1/3 of their daylight hours submerged, and apparently never see dryness. Phenotype differences in the estuary suggest that,
within the habitat, spacing and consequent resource domination may be important parameters affecting plant size and possibly fitness. Nitrogen, which is characteristically rare in this and other estuaries, may be critical in this regard. The plants produce large quantities of glycine-betaine, which may be for simultaneous osmoticum use and nitrogen storage. Most roots occur in the first 3 inches of soil.

A mechanism is proposed, based on highly efficient compartmentation at the cellular level and the shuttling of organic osmoticum across the tonoplast, by which the tidally based cyclical water potentials could be explained.
INTRODUCTION

The genus *Salicornia* contains some of the most salt tolerant Angiosperms in the world (Waisel, 1972). In mixed stands of halophytes, members of the genus typically occupy the most saline sites, capitalizing on their extreme salinity tolerance by colonizing and retaining sites where other species lack the physiology to compete. Inasmuch as it is a standard feature of the genus, the pronounced salinity tolerance probably has a common physiological basis throughout the taxon. While this characteristic is a common attribute of the genus, there is a tremendous range of local adaptation, such that members of the genus occur in a great diversity of saline habitats. Included here are locales which are either constantly wet or generally dry, coastal or inland, tropical, temperate or even arctic, as well as sites of varying degrees of environmental predictability, heterogeneity and intraspecific competition (Wiehe, 1935; Brereton, 1971; Wiebe and Walter, 1972; Ungar, Benner and McGraw, 1979; Jefferies, Jensen and Bazely, 1982; Jefferies and Gottlieb, 1982; Pearcy and Ustin, 1984). A salt marsh is really a mosaic of habitats within which individual species are uniquely adapted. Chapman (1974) reports 26 species of *Salicornia*. Those which have been examined in their native habitat typically show preferences for the specific habitat characteristics associated with the sites in which they occur (Ball and Brown, 1970; Grouzis, 1973;

The subject of this study, the Chenopod Salicornia bigelovii Torr., is an annual member of the genus which occurs exclusively in coastal positions in North and Central America. Along the Pacific coast, the species is first found at Point Conception in southern California. Its intermittent distribution continues southerly on both sides of the Baja peninsula and down the Mexican mainland as far as Mazatlan. Its southernmost distribution along the Gulf of Mexico is the Yucatan peninsula from where it occurs sporadically north to Texas and east to Florida and the Keys in greater abundance. It is also known from several islands in the Caribbean, among them the Bahamas, Jamaica and Puerto Rico. The species also occurs up the eastern seaboard of the United States as far north as New Hampshire (Carolyn Watson, pers. comm.). While the annual reproductive system is atypical of most halophytes, presumably for energetic reasons, it is somewhat more common in halophytic members of the Chenopodiaceae, (Jefferies and Rudmik, 1984). Of course, the annual habit means the plants must recolonize the habitat each year from seed. In the study site at Estero Morua, seed is dehisced into the estuary in late August and September. The seeds germinate quickly over a period of several days and then overwinter as seedlings. Little growth occurs until
March or so when the temperature rises. At this time there is relatively rapid growth for several months. Inflorescence tissue begins to appear in June and develops determinately at each branch end until senescence overtakes the entire plant and seed is once again released into the estuary. Pistils appear prior to stamens as both ascend the inflorescence. It is not known whether this plant is cleistogamous, as are some members of the genus, or whether it is an outcrosser.

Overall, this research assumes the general theory of salinity tolerance in Angiosperms first proposed over a decade ago (Flowers, 1972a, 1972b, 1975; Storey and Wyn Jones, 1975; Flowers, Troke and Yeo, 1977; Storey and Wyn Jones, 1977; Flowers, Hall and Ward, 1978; Wyn Jones, 1979). In this model, \( \text{Na}^+ \) and \( \text{Cl}^- \), which overwhelmingly predominate as the inorganic ions in halophytes, are believed to be sequestered in the vacuole while various species specific enzymatically compatible organic solutes are produced and retained in the cytoplasm as a means of balancing the water potentials between the two compartments. The basis of the model is the discovery that, in vitro, the enzymes of halophytes are no more salt tolerant than those of salt sensitive glycophytes. While this proposed compartmentation has received little direct demonstration (Yeo, 1981), the circumstantial evidence for the model is massive and compelling. An alternative theory, consistent with all the data, has yet to be devised.

It is unlikely, however, that such a simplistic explanation can completely account for the diversity of forms of halophytism or
the variety of habitats occupied by halophytes. In other words, each species will ultimately have to be examined in light of its native habitat in order to fully comprehend the breadth of adaptation at the cellular and whole plant levels. This study is an attempt to examine a particular species in that fashion. Accordingly, this research had two related objectives. The first was to test the effect of salinity on growth and yield in a plant known for extreme salinity tolerance. The second was to understand the results of the first objective with respect to how this species is adapted to the coastal estuary in which it occurs. It was hoped that the realization of these two objectives would to some degree elucidate how the extreme salinity tolerance is achieved and in so doing advance the general model of halophytism through a more detailed understanding of a particular species, *S. bigelovii*, a temperate coastal euhalophyte.

For such an obscure group of plants, the genus *Salicornia* has received considerable attention over the years. This is probably owing to its unique and curious appearance as well as its obvious high tolerance of salinity. Batalin (1876) noticed the requirement for NaCl in order to have normal development, as well as the lack of succulence in the absence of NaCl. Halkett (1915) reiterated Batalin's NaCl requirement for development and added his experimental determination that 1% NaCl (w/v) (i.e. 10 ppt - 10 g. NaCl per 1000 g. solution) was the optimal salinity for growth when *S. ramosissima* was cultivated in its native marsh soil. When grown in nutrient solution, *S. ramosissima* had a growth optimum of between 2% and 3% NaCl while *S. oliveri* grew best at 2%. It is worthy of note that Halkett determined
that *S. oliveri* was unable to flower when grown without NaCl. Barbour (1970) has questioned the obligate nature of any Angiospermous halophyte. Halkett also saw decreasing growth rates with increasing salinities in his experiments.

Nearly 71 years of subsequent experimentation have provided a greatly expanded data base from which essentially the same conclusions are typically drawn for a much larger group of species in the genus. It appears to be nearly universally true that members of the genus have greater growth rates under cultivation conditions which have NaCl concentrations equal to approximately one-third that found in seawater (Langlois, 1967; Barbour and Davis, 1970; Ungar, 1978; Ungar, Benner and McGraw, 1979; Abdulrahman and Williams, 1981; Pearcy and Ustin, 1984).

It was evident from early experiments in this study that the cultivation approach is at least as important a determinant of growth and yield as the salinities selected themselves. Plants grown in hydroponic sand culture experience a pronounced increase in salinity as the NaCl in the nutrient solution accumulates in the sand with successive floodings. The result is the salinities are continually upshifted as the experiment progresses. Unfortunately, this tends to be the approach in most salinity based studies. For this reason, hydroponic water culture was the method of choice in the performance portion of this study as that approach allows for more controlled and stable salinities.

The principal result of this research is that under the experimental conditions described, salinity has virtually no effect on
growth and yield of this euhalophyte. An attempt is made to correlate this finding with observations and plant water relations measurements of the plant made in its native habitat. A mechanism is proposed, consistent with these data and the literature, by which the extreme salinity tolerance could be achieved.
MATERIALS AND METHODS

The Salinity vs. Plant Performance Experiment

The purpose of this experiment was to determine the effect of cultivation salinity on productivity and seed yield as it might be mediated by the water potential, osmotic potential and turgor of the cells.

This experiment had two fundamental components, each of which was served by essentially the same experimental design for growing the plants. In the first part, the intent was to produce plants which would be grown to term at a constant salinity without experiencing other stresses, sampling or disturbance. In an attempt to provide the plants with "unlimited" resources, and thus let salinity be the only potentially limiting variable between treatments, these plants were grown at a range of carefully selected salinities in vigorously aerated hydroponic water culture with abundant nutrients. At the completion of seed filling, and just prior to dehiscence, the plants were harvested and subjected to a harvest analysis.

In the second part, plants were grown specifically for water relations analysis. In this portion of the experiment, the treatments were identical to those in the first part only more plants were grown at each salinity to provide tissue for frequent sampling. Once transplanted into their respective set-ups, the two groups of
treatments were situated adjacent to each other in a greenhouse equipped with thermostatically controlled heating and evaporative cooling. Daytime temperatures were typically in the 80's (F), although the highest daytime summer temperatures were in the mid 90's. The lowest evening temperatures were in the mid 50's.

The germplasm for the experiment was originally collected in Estero Morua, a coastal estuary approximately 4 miles east of Puerto Penasco, Sonora. Initially, on 5/20/85, seeds were sown in the laboratory in two 11 x 22 inch plastic flats lacking drainage. Approximately 1000 seeds, as determined by weighing, were sown in horticultural grade vermiculite in each flat. The flats were positioned 13 cm beneath a bank of 4 ft long Sylvania cool white fluorescent tubes spaced 2 cm apart which provided 210 uE m$^{-2}$ s$^{-1}$ photosynthetically active radiation at the vermiculite surface. During the daylight portion of the 14L/10D photoperiod, the temperature at the vermiculite surface was 32.5 C, while during the night period the temperature was 24.5 C.

To germinate the seeds, the flats were flooded to saturation with 5 liters of distilled water. Within 24 hours, abundant germination was evident. The plants were never allowed to experience drought, which meant watering daily with approximately 600 ml of distilled water. On day 10 after sowing, each flat was watered with 1000 ml of nutrient solution salinized to 2 parts per thousand (ppt - 2 g NaCl to 1000 g distilled water). For the remainder of the time the plants were in the flats, they were watered with distilled water.
Productivity and Yield

On 6/18/85, at 29 days of age, all the plants for the productivity and yield part of the experiment were transplanted into their respective grow out containers. Individuals selected for transplant were of uniform size and appeared healthy. Approximately equal numbers of plants were selected from each flat. A 10 day period was allowed for the replacement of plants which died after the transplant. During that time a total of 14 individuals were replaced. After that period, no dead plants were replaced. This policy resulted in 7 vacancies out of a total of 80 positions at the conclusion of the experiment. The losses appeared to be randomly distributed among the treatments.

These plants were grown with their roots submerged in 5 gallon (18.925 L) white plastic buckets doubly lined with black plastic bags. Each bucket contained 4 plants. The darkness afforded by the plastic bags prevented the growth of algae in the nutrient solution which would possibly cause differences in the availability of nutrients to the Salicornia. Each plant was supported in place by a longitudinally slit foam rubber cylinder 15 mm in diameter which encompassed the stem and was inserted into a hole in the inch thick polystyrene insulation material which served as lids for the buckets. Nutrient solution was a modified Hoagland’s formulation as described in Appendix A. Half strength nutrients were re-added to each treatment at the beginning of each month. At midday on a typical sunny day, the plants received a peak of 1650 uE m⁻² s⁻¹ photosynthetically active radiation.
The salinity treatments selected were 1, 10, 35, 45 and 60 ppt prepared as g Instant Ocean per liter of nutrient solution. These values correspond to water potentials of -0.07, -0.77, -2.72, -3.50 and -4.67 MPa respectively, while seawater is about -2.3 MPa. Instant Ocean is a commercial seawater preparation (Aquarium Systems, Mentor, Ohio). For each treatment there were 4 replicates, each containing 4 plants. Hence, there were initially 16 plants in each treatment. Distilled water was used for the nutrient solution for the 1 ppt treatments, while tap water was used for all the other salinities.

Solutions were stirred until all solutes had dissolved. During the course of the experiment, the containers were largely without precipitates. As the experiment proceeded, the salinity of each container was periodically monitored by conductivity with a Markson Electro Mark conductivity meter (Markson Science Inc., Del Mar, Ca.). Any salinity deficiency was corrected by the addition of an appropriate amount of Instant Ocean. Due to transpirational losses, water was added to the containers to maintain the proper volume. Initially, when the plants were small, the amount was insignificant. When the plants became sizable, the containers required daily additions of about 2 liters of water. The salinized nutrient solution was continuously aerated.

On 12/10/85, at 205 days of age, when all plants had completed seed filling yet had not dehisced the seed, harvesting was begun. At this time, the plants were dead and had dried as far as atmospheric conditions would allow. They were, in effect, standing dry weight. With extreme care, so as to not lose any material, each individual was
harvested and divided into 3 categories: 1. seed, 2. inflorescence tissue minus the seed, and 3. branches and the main stem. Only shoots were collected. The separation of the material was fairly simple with the exception of cleaning the seed of inflorescence tissue. As is typical of the Chenopodiaceae, each locule contains one ovule. In S. bigelovii each locule has a cover which in the wild falls away at maturity releasing the seed. Under the conditions in which these experimental plants were grown, the locule covers were not dislodged. Consequently, the inflorescence tissue had to be broken up by lightly grinding the material on a seed grinding board. Differential sifting of the material through a range of screens served to separate the bulk of the chaff from the seeds. Finally, a means of winnowing the material was devised to separate the seed from chaff particles of the same size. The seeds are sufficiently denser than identically sized chaff particles such that this step resulted in very clean seed. After cleaning, the seeds from each plant were weighed on a Mettler AE 160 balance. Weights of the other categories were determined with a Sartorius 1202 MP top loading balance.

The percent ash of the different plant parts was determined by ashing the material for 4 hours at 550 C in a Lindberg Hevi-Duty muffle furnace (Watertown, Wisc.). In order to obtain a true dry weight, and therefore a true ash weight and percentage, samples were dried for 4 hours at 135 C in a VWR model 1370 drying oven prior to ashing. After drying, samples were cooled in a dessicator and dry weights were taken at room temperature. Samples were then ashed in porcelain crucibles which were thoroughly cleaned with 20% nitric acid
(v/v) before each use. Again, the ash was cooled in a dessicator and weighed at room temperature. All weighings for the ash determinations were done to four decimal places on the Mettler AE 160 balance.

Tissue glycine-betaine content was assayed by a slight modification of the method of Stumpf (1984) wherein the background absorbance of the modified Dragendorf's reagent was estimated by assaying 2 blank 1.8 ml microfuge tubes treated with the reagent as the samples had been.

Where appropriate, 95% confidence intervals have been calculated as 2 standard errors above and below the mean.

Water Relations

**Cell Osmotic Potential.** Attempts to determine the osmotic potential of the cells by psychrometry met with little success. Turgors calculated as the difference between water potential and osmotic potential were generally negative, a result inconsistent with the appearance and growth of the plants. This is not an uncommon situation with some plant tissues. It is the consequence of underestimating the osmotic potential of the cells due to the mixing of the relatively dilute solution from the apoplasm with that of the symplasm which represents the true osmotic potential of the cell. The mixing occurs when the psychrometer chamber containing the tissue is immersed in liquid nitrogen so as to rupture the plasmalemma and tonoplast and eliminate cell turgor.
Attempts to determine the cell osmotic potential with the pressure bomb in the manner of Tyree and Hammel (1972) were also unsuccessful. *S. bigelovii* tends to generate fairly low cell osmotic potentials in any cultivation salinity, thus requiring substantial bomb pressures for a pressure/volume curve. Unfortunately, the succulent nature of the tissue precludes the application of such pressures.

Finally, it appeared as though the only means of obtaining the osmotic potential of the cells was the technique of plasmolysis. Accordingly, the procedure detailed in Appendix B was devised based on the general approach of Barrs (1968).

**Bulk Tissue Water Potential.** Bulk tissue water potentials were measured with model 75-13C thermocouple psychrometers (J. R. D. Merrill Specialty Equipment Co., Logan, Utah). Each psychrometer was individually calibrated prior to use with sodium chloride solutions. Periodically, all psychrometers were recalibrated. Inasmuch as contamination can lead to measurement errors, each psychrometer was thoroughly cleaned after each use by spraying with LPS contact cleaner, and then rinsing well with distilled water and acetone.

Water potential samples were taken by excising the first 3 to 4 mm of the shoot tip with a fresh blade and immediately sealing the tip in a psychrometer chamber. During the equilibrium period, psychrometers were maintained at a constant 24.5 ± 0.1 °C temperature. The temperature of the water bath was checked frequently during the equilibrium process to ensure thermal stability. At the end of the 4 hour equilibrium period, each psychrometer was read in the dewpoint
mode using a model HR-33T Microvoltmeter (Wescor, Inc., Logan, Utah). Subsequently, microvolt readings were converted to water potential values using the particular pre-determined conversion factor for each individual psychrometer.

**Cell Turgor.** As is historically the case, turgor was calculated as the difference between water potential and osmotic potential rather than empirically determined (Barrs, 1968).

**Experiments in Estero Morua, Puerto Penasco, Sonora**

To complement the laboratory and greenhouse studies, field studies were conducted. Accordingly, two trips were made, one from 7/3 to 7/7/85 and the other from 8/14 to 8/16/85, to Estero Morua just east of Puerto Penasco, Sonora, Mexico, where the original germplasm had been collected (Figs. 1 and 2). From the air, the estuary appears as a maze of channels and vegetation (Fig. 3).

The first trip had two basic objectives: (1) to determine where in the tidal range as depicted on the 1985 Tide Calendar for the Northern Gulf of California (Univ. of Arizona, Tucson, Arizona) the plants occur, and (2) to determine if bulk tissue water potential changes as the plants experience the tidal cycle. The answers to both of these questions have important implications in understanding how the plant operates in the wild. Additionally, various other data were collected among which were the following determinations: (1) maximum root depth as well as overall root morphology, (2) rate of tidal movement, (3) low tide soil water potentials at 3, 6 and 12 inches
Fig. 1. Map of the northern Gulf of California. As part of a wider distribution, *S. bigelovii* occurs sporadically along the coastline shown. The germplasm for this study was originally collected in Estero Morua, approximately 6 km southeast of Puerto Penasco. After Yensen (1979).
Fig. 2. Map of Estero Morua. The estuary is 8 km long by nearly 2 km wide at its widest point. During high tides these dimensions can expand considerably. The numbers along the northern shore indicate the study sites described in the text.
Fig. 3. Aerial view of the western portion of Estero Morua. This photo, taken at low tide, shows the major channels in this part of the estuary. During high tides, the land between the whitish areas of the foreground and background is entirely submerged. During low tide, the water in the main channel is ankle deep, but at high tide can be 25 feet deep.
depth, (4) % ash and % ash free dry weight of vegetative and reproductive parts of wild plants, and (5) water potential of several individuals growing in the estuary channel per se (site 3) rather than more along the shore where virtually all the *S. bigelovii* occur.

The objectives of the second trip were essentially the same as the previous outing only with sampling for elevation and high and low tide plant water potentials done over a much wider area so as to substantiate the conclusions as species characteristics in the estuary at large. The techniques for measuring these parameters were the same as those of the previous trip. Also, a substantial portion of the *S. bigelovii* population was surveyed to determine the extent of the species' occurrence in the estuary. Further observations about the species' overall ecological position in the estuary were made as the opportunity arose.

Elevation of the Soil Surface Relative to the Tide Chart

As a means of determining the elevation of the soil surface at site 1, and therefore of the plants relative to the tide chart, 2 ft of a 10 ft piece of PVC pipe was driven into the soil at the exact location where the plant and soil water potential sampling had been done. The pipe was delineated into 6 inch intervals. Actual high tide was determined by observing the high water mark of the incoming tide and noting the corresponding height of the water on the pipe. The position of the soil surface on the tide chart is the high tide reading at that high tide minus the depth of water as read on the
pipe. The pipe was marked at the 2 ft depth into the soil and this mark was checked during the course of the determination to ensure that the pipe did not change its position relative to the soil. Soil surface elevations at the other sites were determined by noting the time at which the surface began to be submerged by the incoming tide and checking the tide chart for the elevation of the tide at that time.

High and Low Tide Bulk Tissue Water Potential

"High tide" values were taken when the tide had receded to the point where the soil surface was just emerging. "Low tide" values were obtained when the tide was rapidly coming in and was still several feet below the soil surface. These sampling times were designed to magnify any possible water potential differences.

Plant water potential was determined as follows: A healthy looking shoot tip (all inflorescences) was selected, rinsed generously with distilled water from a squirt bottle, gently blotted dry with a Kimwipe, and allowed to air dry for about a minute. Each tip was carefully inspected for surface water before cutting and loading. Hands were dried thoroughly for the cutting and loading steps as well. Then, the last 5 mm of the shoot tip was excised with a sharp blade and immediately sealed in a psychrometer chamber. The psychrometers were incubated for 4 - 16 hours at 27.5 C in water in a styrofoam container in a beach house. Finally, the psychrometers were read using the Wescor HR-33T microvoltmeter powered by fresh batteries.
Additional Data

Soil water potential samples were collected by driving a long bladed shovel into the soil and turning a spadeful over. The edge of the hole was then quickly squared off. Immediately then, a punched disc of Whatman no. 1 filter paper was saturated by contact with the soil at each depth and quickly sealed in a psychrometer chamber. They were incubated and read in the same fashion as the plant samples.

Maximum root depth as well as overall root morphology were evaluated by carefully digging several plants out and gently washing the soil off in the water in the channel. The soil is cake-like and came apart in clumps snapping the slender roots at the 6 to 8 inch length. However, a few plants were successfully washed clean of the soil and showed roots which were uniformly threadlike at the tips. Gross root morphology was recorded with 35 mm slides.

The rate of tidal movement was determined by inserting two sticks into the soil, finding their elevational difference with the use of a level and a tape measure, and recording the time required for the water level to move from the base of the lower stick to that of the higher one.

The percent ash and percent ash free dry weight of vegetative and reproductive parts of these wild plants were determined by collecting samples just prior to returning to Tucson, rinsing off any surface salt with distilled water, drying them at 135 C in the drying
oven on arrival, and ashing them in the muffle furnace at 550 °C for 4 hours. The technique was identical to that for the lab grown tissue.

Particle size and nitrate analysis of the soil from Estero Morua was performed by the staff of the Fleischman Lab at the Environmental Research Lab. Soil particle size distribution was determined according to Day (1965). Nitrate extraction was according to Bremner (1965).

Additionally, the Tide Calendar, which contains a wealth of useful information, has been used as a source of environmental data.

Additional Experiments

During the course of this work, numerous ancillary experiments were performed to refine the emerging understanding of the plant. These analyses provide useful insight into some of the more subtle aspects of the plant's adaptations to the estuarine environment. Several of those experiments which seem particularly relevant are briefly recorded below.

1. One experiment involved evaluating the rate and bidirectionality of water potential adjustment. This was done by sowing seeds in horticultural grade vermiculite in two 1000 ml plastic beakers fitted with a corked hole in the bottom for controlled drainage. Initially, the seeds were germinated with distilled water. To change the rhizosphere salinity, each container was perfused with a solution of the new salinity by filling it to saturation and then draining by the removal of the cork. The drained solution was
discarded each time and new solution was used with every pass of the new salinity through the system. Every time the salinity was changed this procedure was repeated 10 times. Finally, the cork was replaced again and the beaker filled with nutrient solution salinized to the new level. Water potential samples were taken either 12 or 24 hours after salinity changes. Salinities were moved up and down and then up again to see how well the plants would track the water potential of the solution. Twelve psychrometers were used for each determination, each sampling a different seedling.

2. During the course of some work on the water relations of seedlings, extensive determinations were made of the water potential of seedlings germinated in 14 cm petri plates at 0, 10 or 30 ppt. The seeds were originally irrigated to saturation with about 150 ml of one of those treatments and subsequently watered daily with approximately 50 ml of distilled water to return the plates to their original saturation. An identical set of unseeded plates was prepared and monitored by weighing for the salinity changes associated with the daily water loss.

3. During an early trip to Estero Morua in June of 1983, several wild plants from the estuary were collected for glycine-betaine analysis. Four plants were analysed. Two of them were divided into root, senescent appearing shoot, non-senescent appearing shoot, and inflorescence. The other two were analysed in the same fashion, only without the roots. As with the lab grown material, the assay was done according to the method of Stumpf (1984).
4. Of several attempts to grow plants to term at a range of salinities, one experiment in particular produced a serendipitous result. This experiment involved growing the plant at 0, 5, 10, 20, 30, 35, 40, and 50 ppt in hydroponic sand culture. The unexpected event was the spontaneous abortion of virtually all the seeds in the 20 ppt treatment. As usual, the 0's did not survive long enough to reproduce, nor, surprisingly did the 50's. All the other treatments, save the 20's completed the life cycle producing the 1.0 mg seed typical of this ecotype. The 20's had formed seed but the seeds were unfilled.
RESULTS

Productivity and Yield

Copious transpiration combined with effective salt absorption by the plants resulted in the treatments being approximations of their intended values (Fig. 4). The treatments remained discrete from one another throughout the course of the experiment, although in some cases the salinities, as determined by conductivity measurements, were less than the specified values. Conductivity samples were collected when the containers were full and well mixed, so the samples are indicative of the lowest salinity the roots experienced. In effect, then, the sampled values represent the closest the treatments came to one another. This cyclical effect is inherent in the experimental design and approximates the same phenomenon in Estero Morua. Therefore, these results represent the effects of a wide diversity of fairly stable, yet cyclical, salinities, not unlike what occurs in the wild.

The most striking result is the complete lack of any significant variation in shoot dry weight between the four most saline treatments (Fig. 5). The mean shoot dry weight for all of them was between 55.4 and 62.4 g. The lowest mean, that of the 60's, is 88.8% of that of the 35's, which is the highest (Table 1). For these four treatments, not only are the means not significantly different, but
Fig. 4. Conductivity determined salinities in the performance experiment.
Fig. 5. Effect of salinity on shoot dry weight. The only significant differences were the l's, which were significantly different from all other treatments. Error bars represent 95% confidence intervals. Sample size (n) is given at the base of each error bar.
Table 1. Means, 95% confidence intervals and % of highest yielder of shoot dry weight, seed weight per shoot and seed weight/shoot weight of S. bigelovii at 5 salinities. 95% confidence intervals are 2 standard errors above and below the mean. Salinities are ppt Instant Ocean.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>10</th>
<th>35</th>
<th>45</th>
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<td>Shoot dry wt</td>
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<td>57.15</td>
<td>62.41</td>
<td>57.84</td>
<td>55.40</td>
</tr>
<tr>
<td>95% interval</td>
<td>20.96-35.20</td>
<td>49.35-64.95</td>
<td>49.82-74.99</td>
<td>48.61-67.09</td>
<td>43.30-67.50</td>
</tr>
<tr>
<td>% of highest yield</td>
<td>44.99</td>
<td>91.57</td>
<td></td>
<td>92.68</td>
<td>88.77</td>
</tr>
<tr>
<td>Seed wt per shoot</td>
<td>4.872</td>
<td>11.619</td>
<td>11.161</td>
<td>11.629</td>
<td>10.785</td>
</tr>
<tr>
<td>% of highest yield</td>
<td>41.89</td>
<td>99.92</td>
<td>95.98</td>
<td></td>
<td>92.74</td>
</tr>
<tr>
<td>Seed wt/shoot wt</td>
<td>0.170</td>
<td>0.202</td>
<td>0.176</td>
<td>0.199</td>
<td>0.190</td>
</tr>
<tr>
<td>95% interval</td>
<td>0.132-0.208</td>
<td>0.189-0.216</td>
<td>0.158-0.193</td>
<td>0.187-0.209</td>
<td>0.172-0.208</td>
</tr>
<tr>
<td>% of highest yield</td>
<td>84.15</td>
<td></td>
<td>86.67</td>
<td>98.17</td>
<td>93.88</td>
</tr>
</tbody>
</table>
the 95% confidence intervals are nearly completely overlapping. During the period these plants were growing, differences in growth rate were undetectable, and the plants appeared indistinguishable. Curiously though, each treatment, including the 1's, had one or two individuals which either lagged far behind or well outperformed all remaining plants in that treatment. This has the effect of expanding the confidence intervals. Figs. 6 - 10 show the common performance of the four highest salinity treatments. The lack of any differences among them is particularly evident in Fig. 11. Additionally, there were no obvious variations in gross morphology between plants in the four higher salinities.

The 1 ppt treatment did not perform nearly as well as the other salinities (Figs. 5, 6 and 11). In fact, the mean shoot dry weight at 28.1 g was significantly different at the 95% confidence level, being just about half of the common value of each of the other treatments. The confidence interval was only slightly smaller for the 1's than for the other salinities. During the course of the experiment, the 1's had a more compact appearance and were a much darker green. Interestingly, this treatment displayed reproductive activity about two weeks prior to the other treatments which behaved in concert.

The data pattern regarding mean seed weight per shoot was virtually identical to the shoot dry weight results (Fig 12 and Table 1). The four highest salinities exhibited a common yield of about 11 grams of seed per plant. Again, of these four, the mean of the lowest yielding treatment, the 60's, was 92.7% of that of the highest, the
Fig. 6. 1 ppt plants. The vacancy represents an individual which did not survive. The plant in the foreground was unusually stunted, even for a 1 ppt individual. The plant on the left was a typical 1 ppt plant, while the one in back of the sign was exceptionally large.
Fig. 7. 10 ppt plants. These plants were the lush, highly succulent phenotypes typical of 10 ppt individuals.
Fig. 8. 35 ppt plants. During the period these plants were growing, they were indistinguishable from the 10's. Ten ppt is often cited as the optimal salinity for growth.
Fig. 9. 45 ppt plants. As with the 35's, these plants had the same appearance as the 10's.
Fig. 10. 60 ppt plants. The slightly smaller stature of these plants relative to the 10's was barely detectable. As with the plants in the three higher salinities, these individuals lacked the highly compact appearance often associated with those salinities.
Fig. 11. The 5 salinity treatments. From left to right, the treatments are 1, 10, 35, 45 and 60 ppt. The 1's are markedly smaller than the rest, while the other treatments appear indistinguishable.
Fig. 12. Effect of salinity on mean seed weight per shoot. As with the shoot dry weights, only the 1's were significantly different from any other treatment. Error bars represent 95% confidence intervals. Sample size (n) is given at the base of each error bar.
45's. The confidence intervals show the same variability, making the treatments indistinguishable.

There is no obvious discernable pattern in the slight variation among the four highest salinities. The 60's do have marginally less shoot dry weight and seed weight, but the best dry weight performance is that of the 35's, while the 45's have the greatest seed yield. All the differences are, however, slight. The variation within a treatment far exceeds that between their means (Figs. 5 and 12).

As was the case with the dry weights, the seed yield of the 1's, at just under 5 g, was significantly different from the common yield of the four other salinities. It was, however, in the same proportion such that the mean of seed weight/shoot dry weight was the same for all five treatments at 0.17 to 0.20. In other words, 17 to 20% of the shoot dry weight is seed. Overall, the 1's had 42% of the seed weight and 45% of the shoot dry weight of the other treatments (Table 1).

The ash content largely reflects the performance data (Fig. 13). For the inflorescence tissue, once again, the 1 ppt treatment stands out from the rest of the salinities in having less ash than the regression line for the other salinities would predict. However, the discrepancy is not as great as with the performance data. The percent ash of the 1's is 34.6%, a value not far below the others. The percent ash of the four higher salinities extends from 42.2% at 10 ppt to 50.9% at 60 ppt, a fairly narrow scope considering the range of the treatments. The small slope of the regression line is indicative
Fig. 13. Effect of salinity on percent ash of three plant parts. Inflorescence tissue typically has the greatest ash percentage, followed by branch and main stem. In all cases, the l's had lower percentages than the regression lines predict. Regression lines do not include the 1 ppt points. Error bars represent 95% confidence intervals.
of the modest differences in the percent ash of the inflorescence tissue. Overall, the ash content of all the treatments seems rather large, with the 1's having nearly 35% of their dry weight as absorbed inorganic ions. At the other end of the scale, the 60's are just under 51% ash, which, while a substantial amount, seems like not that much more than the 1's. In this tissue, a sixty-fold increase in cultivation salinity produces a 1.46 fold increase in ash content.

The data are similar for the branch tissue, although the slope of the regression line is greater indicating a more pronounced change in ash content with cultivation salinity. The 45's and 60's have virtually the same ash content as their inflorescence counterparts. The 10's and 35's, however, have significantly lower amounts of ash than the corresponding inflorescence tissues. Again, the 1's lag behind all other treatments in having an ash content which is less than the regression line for the other treatments would predict.

The main stem has a uniformly low percent ash in all of the treatments. Here, the 1's seem to be in line with the other salinities, as the mean for that group very nearly falls on the regression line for the others. The range for all the treatments is a relatively low 6.6 to 12.7% ash.

Interestingly, out of a total of 219 ashings which were done (73 plants x 3 harvest categories each), 41 of those determinations (18.7%) seemed anomolous. Most of these "anomalies" were only moderately so, but some appeared more extreme. As these values were suspect, each was re-ashed. In the overwhelming majority of cases (70.7%), the second value was essentially the same as the first,
differing only by 1 per cent or less. In the remainder of the samples (29.3%), the values differed by more than 1%, with a few larger differences of 10 - 15%.

Analysis of the glycine-betaine content of the inflorescence tissue shows the l's, at 242.7 umoles g\(^{-1}\) AFDW, to have significantly more organic osmotica than any of the other treatments (Fig. 14). The remaining treatments display no easily discernable pattern. The 10's and 35's have nearly identical glycine-betaine contents at 153.4 and 151.0 umoles g\(^{-1}\) AFDW, respectively. The 45's are unexpectedly low with a mean of 102.7, while the 60's have virtually double that amount at 201.9 umoles g\(^{-1}\) AFDW. Once again, however, the l's seem to be distinct from the other salinities, only this time in excess. The l's are significantly different from all the other treatments except the 60's.

The branches have a glycine-betaine content which varies directly, although only moderately, with cultivation salinity. The l's have 42.4 umoles g\(^{-1}\) AFDW, which is 47.6% of the 88.97 umoles the 60's have. This is a 2.1 fold increase with a 60 fold increase in cultivation salinity. The regression line for the branches fairly closely parallels that of the inflorescence material, as the similar slopes of the equations indicate. The branches do contain significantly less glycine-betaine than the inflorescences produced in the same salinity.

The main stem of the plant contains not only the least glycine-betaine of any of the three plant parts, but the amount varies only marginally with salinity. Over the course of a 60 fold increase
Fig. 14. Effect of salinity on glycine-betaine content of three plant parts. As with the ash content, the inflorescence has the greatest complement of glycine-betaine, followed by the branch and main stem. The l's have the greatest inflorescence glycine-betaine content of all the treatments. Regression lines do not include the l ppt points. Error bars represent 95% confidence intervals.
in cultivation salinity, the increase in glycine-betaine content is 1.3 fold. That is, the 1's have 76.6% of the amount the 60's have.

In spite of the limited resolution of the inflorescence points, the glycine-betaine in the plant does appear to be distributed unequally in these three categories. In the four higher salinities, the inflorescences have 2 to 3 times the glycine-betaine of the branches, while the ratio for the 1's is nearly 6 to 1. In the 1's, the glycine-betaine content of the inflorescences is concentrated almost 10 times beyond that of the main stem. For the other treatments, the inflorescences have approximately 5 times the glycine-betaine content of the main stems.

The glycine-betaine distribution pattern among the three plant parts for wild plants from the estuary (Fig. 15) is identical to that of the greenhouse grown plants (Fig. 14), although the wild plants have markedly higher levels. Roots tend to have the least glycine-betaine, followed by the senescent portion of the shoot, then the nonsenescent shoot and finally the inflorescence which has far and away the most.

In another group of plants grown to maturity under similar conditions and salinity levels, for an unknown reason the seeds in the 20 ppt treatment aborted. Analysis of the glycine-betaine levels in the plants in each treatment provided some interesting results. The 20 ppt treatment in which virtually all the seeds were aborted shows extremely high residual levels of glycine-betaine in the inflorescence tissue, although the levels in the branch and main stem are similar to the low levels in the other treatments (Fig. 16). The 40's, which as
Fig. 15. Glycine-betaine content of various parts of S. bigelovii from Estero Morua. The distribution pattern is the same as in the greenhouse grown plants, although the amounts in each plant part are several fold greater. When sampled, these plants were in the seed filling stage.
Fig. 16. Effect of unfilled seeds on the glycine-betaine content of the inflorescence. Seeds in all the treatments except the 20's were filled and appeared normal size. Very few seeds in the 20's were filled to any degree at all. This resulted in unusually high amounts of glycine-betaine remaining in the inflorescence in that treatment.
the highest salinity should have the greatest levels of glycine-betaine, have the second highest glycine-betaine content, that being only 64.5% of the amount present in the inflorescence of the 20's.

**Water Relations**

The treatment cultivation salinities were carefully monitored before the tissue was sampled to ensure the determinations would be representative of the intended salinities. In reality, those salinities were very close to, although not exactly, the named values of the treatments. In all cases, the differences are slight and inconsequential (Table 2).

In each treatment, the plants were able to generate water potentials which were more negative than those of the salinized nutrient solutions, thus establishing a gradient for water import (Table 2 and Fig. 17). The smallest of these gradients, 0.83 MPa, was that of the 60's. The largest gradient, 1.20 MPa, occurred in the 10 ppt treatment. Even the 1 ppt treatment, which routinely performed differently from the other treatments, established a gradient of 0.92 MPa. The regression equation calculated for the four higher salinities shows a small, but distinct, decrease in the gradient with increasing salinity. The high correlation coefficient (0.999) lends credence to that trend. The regression equation which includes the 1's has a correlation coefficient (0.997) which is barely lower than
Table 2. Water relations of *S. bigelovii* at 5 salinities. Salinities are in ppt Instant Ocean. Water potential, osmotic potential and turgor are in MPa.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>1</th>
<th>10</th>
<th>35</th>
<th>45</th>
<th>60</th>
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<tr>
<td>Actual salinity</td>
<td>1.4</td>
<td>8.8</td>
<td>34.4</td>
<td>43.8</td>
<td>56.4</td>
</tr>
<tr>
<td>Nutrient solution $\psi$</td>
<td>-0.10</td>
<td>-0.68</td>
<td>-2.67</td>
<td>-3.40</td>
<td>-4.39</td>
</tr>
<tr>
<td>$\psi$</td>
<td>-1.02</td>
<td>-1.88</td>
<td>-3.78</td>
<td>-4.39</td>
<td>-5.22</td>
</tr>
<tr>
<td>$\psi$ gradient</td>
<td>0.92</td>
<td>1.20</td>
<td>1.11</td>
<td>0.99</td>
<td>0.83</td>
</tr>
<tr>
<td>$\psi_\Pi$</td>
<td>-1.13</td>
<td>-2.71</td>
<td>-4.45</td>
<td>-4.87</td>
<td>-5.83</td>
</tr>
<tr>
<td>$\psi_p$</td>
<td>0.11</td>
<td>0.83</td>
<td>0.67</td>
<td>0.48</td>
<td>0.61</td>
</tr>
</tbody>
</table>
Fig. 17. Water potential, osmotic potential and turgor of *S. bigelovii* at 5 salinities. Water potentials were determined by psychrometry, osmotic potentials by plasmolysis and turgor by difference. The maintenance of turgor at high salinity reflects the performance data where growth and seed yield were undiminished. The regression lines do not include the 1 ppt points. Error bars represent 95% confidence intervals.
that of the equation which excludes them. Of note here is not only
the moderately declining gradients, but the very wide range of water
potentials of which the plant is, apparently, easily capable. Also,
with the exception of the 1's, the 95% confidence intervals expand
with increasing salinity. The values for those intervals are 0.16,
0.20, 0.26 and 0.48 MPa respectively for the 10's, 35's, 45's and
60's. The value for the 1's, at 0.36 MPa, occurs between those of the
45's and 60's.

Analysis of the 35 mm slides produced Fig. 28 in Appendix B
depicting the relationship between the percent plasmolysis and the
osmotic potential of the incubation solution for a particular
treatment. Table 3 lists the calculated regression equations,
correlation coefficients and points of incipient plasmolysis. The
plasmolytically determined osmotic potentials show a trend that is
fundamentally the same as the water potentials. The most obvious
characteristic is that once again, osmotic potential decreases
linearly with increasing salinity. The correlation coefficient of
0.998 suggests this is a dependable observation. The 1's have an
osmotic potential which is higher than the prediction of the
regression line (Fig. 17). As was seen with the water potential
grades, with the exception of the 1's, for a given treatment, the
difference between the cell osmotic potential and the water potential
of the cultivation solutions narrows with increasing salinity. These
values are 1.03, 2.03, 1.78, 1.47 and 1.44 MPa respectively, for the
1's, 10's, 35's, 45's and 60's. Additionally, with the exception of
the 45's, the slopes of the lines in Fig. 28 decrease with increasing
Table 3. Plasmolysis regression equations, correlation coefficients (r) and points of incipient plasmolysis (PIP) at 5 salinities.
Salinities are in ppt Instant Ocean. Points of incipient plasmolysis are in MPa.

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>r</th>
<th>PIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>y = -16.189x + 31.753</td>
<td>-0.979</td>
<td>-1.127</td>
</tr>
<tr>
<td>y = -12.693x + 15.559</td>
<td>-0.954</td>
<td>-2.714</td>
</tr>
<tr>
<td>y = -10.110x + 4.964</td>
<td>-0.926</td>
<td>-4.454</td>
</tr>
<tr>
<td>y = -12.191x - 9.355</td>
<td>-0.949</td>
<td>-4.867</td>
</tr>
<tr>
<td>y = -7.104x + 8.566</td>
<td>-0.922</td>
<td>-5.833</td>
</tr>
</tbody>
</table>
cultivation salinity, meaning that the per cent increase in plasmolysis with a given increment in incubation osmotic potential is less in plants grown at higher salinities. The 45's are slightly out of conformity with this observation in having a slope that is just 2.08 greater than that of the 35's.

The calculated turgors (Table 2 and Fig. 17) show the same trend generally seen in the other data. The turgor for the 1's is quite low at 0.11 MPa, while the other treatments seem once again to be grouped together having turgors of 0.83, 0.67, 0.48 and 0.61 MPa for the 10's, 35's, 45's and 60's, respectively. Overall, there appears to be a small decrease of turgor with increasing salinity, although the 60's are somewhat out of line with the trend. The small positive slope of the regression equation indicates the slightly declining nature of the turgor at increasing cultivation salinities.

Plants grown in the 14 cm petri plates at either 0, 10 or 30 ppt arrive at a characteristic water potential by about day 6 and remain at that water potential as long as the plates remain in the same cycle (Fig. 18). The water potential of the vermiculite in each treatment stays within narrow bounds as long as the plates are promptly rewatered each morning (Fig. 19). Fresh plates newly sown with seed were continually set up in order to provide tissue of a particular age and salinity. Inherent in this approach is the variability between replicates which probably accounts for much of the variability in the water potential values within a treatment.

The plants can depress their water potential quite rapidly, although increases in water potential take longer (Fig. 20). Sudden,
Fig. 18. Effect of age on water potential at 4 salinities. Seeds were sown in vermiculite in 14 cm petri plates. Until about day 20, only cotyledons are available for sampling. The last few samples were meristematic, indicating no appreciable differences in water potential between the two tissue types. Within a given salinity cycle, plants develop a characteristic water potential. Each point represents a minimum of 10 samples.
Fig. 19. Cyclical water potential of 14 cm petri plates. In an identical fashion to the plates used for the plants in Fig. 18, these plates were initially irrigated with a particular salinity and subsequently rewatered each morning with distilled water. As determined by weighing, just prior to rewatering the -0.77 MPa (10 ppt) treatment becomes -1.10 MPa. The -1.56 MPa (20 ppt) treatment becomes -2.17 MPa. The -2.34 MPa (30 ppt) treatment becomes -3.34 MPa. Higher cultivation salinities produce cycles of greater amplitudes.
Fig. 20. Bidirectional water potential adjustment. The plant can decrease its water potential rapidly, but increasing it takes longer. Presumably, this is because decreasing water potential is accomplished largely by substantial ion uptake, such that at high soil water potentials the plants are unable to rid themselves of accumulated ions.
extremely low environmental water potentials may require longer periods for osmotic adjustment than more moderate changes.

**Experiments in Estero Morua**

The data from Estero Morua can be grouped into three categories: 1. plant and corresponding soil water potentials, 2. site elevation determinations relative to the tide chart, and 3. a variety of other measurements, notes and observations. The first two groups are summarized in Table 4 which includes the data from both trips to the estuary.

Plant water potentials were sampled at numerous sites, each of which merits a brief description. Site 1 is on the mudflats about 200 yards east of where the road first meets the estuary (Fig. 2). The area varies from about 40 - 100 yards wide and the length runs out of sight along the shore. At one particularly wide point there is a slight promontory which is densely populated with *S. bigelovii*. This specific location is site 1 (Fig 21). Site 2 was a designated area which received little attention or sampling.

Site 3 is about 300 yards west of site 1 along a narrow band of shore. It is actually a composite of three distinct sampling locations close enough in proximity to share a common site designation. The basis of this distinction was the three very obviously different phenotypes in the three subsite positions. While one subsite was a shore position, two of the subsites were located in the channel itself, not along the shore as all the other sites were.
Table 4. Water potentials and site elevations of *S. bigelovii* with corresponding soil water potentials in Estero Morua. All plant samples are 5 mm inflorescence tips. Site elevations are according to the 1985 Tide Calendar for the Northern Gulf of California. Water potential values are means in MPa. Soil water potentials were determined at 3, 6 and 12 inches depth. Values are recorded in that order. 95% confidence intervals are 2 standard errors above and below the mean.

<table>
<thead>
<tr>
<th>Date</th>
<th>Site</th>
<th>Site elevation</th>
<th>Tide type</th>
<th>Sample type</th>
<th>$\psi$</th>
<th>95% interval</th>
<th>n</th>
</tr>
</thead>
<tbody>
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<td>9' 2'' high</td>
<td>plant</td>
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<td>-4.14 - -4.38</td>
<td>13</td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>9' 2'' low</td>
<td>plant</td>
<td>-4.69</td>
<td>-4.49 - -4.89</td>
<td>7</td>
<td></td>
</tr>
<tr>
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<td>9' 2'' low</td>
<td>soil</td>
<td>-4.12</td>
<td>-3.35 - -2.24</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7-4-85</td>
<td>1</td>
<td>9' 2'' low</td>
<td>soil</td>
<td>-3.76</td>
<td>-0.41 - -3.58</td>
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<td></td>
</tr>
<tr>
<td>7-5-85</td>
<td>1</td>
<td>9' 2'' low</td>
<td>soil</td>
<td>-4.12</td>
<td>-3.96 - -4.28</td>
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</tr>
<tr>
<td>7-5-85</td>
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<td>6' 0'' a*</td>
<td>plant</td>
<td>-4.06</td>
<td>-3.66 - -4.46</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8-14-85</td>
<td>3</td>
<td>6' 0'' high</td>
<td>b*</td>
<td>-0.45</td>
<td>-0.80 - -3.42</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8-14-85</td>
<td>3</td>
<td>6' 0'' high</td>
<td>soil</td>
<td>-4.79</td>
<td>-4.71 - -4.87</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>8-14-85</td>
<td>3</td>
<td>6' 0'' high</td>
<td>c*</td>
<td>-4.21</td>
<td>-3.71 - -3.45</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8-14-85</td>
<td>3</td>
<td>10' 6'' high</td>
<td>d*</td>
<td>-4.12</td>
<td>-3.82 - -4.42</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
### Table 4, Continued

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Height</th>
<th>Type</th>
<th>Soil 1</th>
<th>Soil 2</th>
<th>Soil 3</th>
<th>Notes</th>
</tr>
</thead>
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<tr>
<td>8-14-85</td>
<td>3</td>
<td>10' 6&quot;</td>
<td>high</td>
<td>-3.66</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>8-15-85</td>
<td>5</td>
<td>9' 6&quot;</td>
<td>high</td>
<td>-4.59</td>
<td>-4.53</td>
<td>-4.65</td>
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<td>8-15-85</td>
<td>6</td>
<td>N.D.</td>
<td>high</td>
<td>-5.22</td>
<td>-5.12</td>
<td>-5.32</td>
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<td>8-15-85</td>
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<td>N.D.</td>
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<tr>
<td>8-15-85</td>
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<td>N.D.</td>
<td>high</td>
<td>-4.02</td>
<td>-3.74</td>
<td>-4.30</td>
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<tr>
<td>8-16-85</td>
<td>1</td>
<td>9' 4&quot;</td>
<td>low</td>
<td>-5.14</td>
<td>-4.82</td>
<td>-5.46</td>
<td>8</td>
</tr>
<tr>
<td>8-16-85</td>
<td>3</td>
<td>6' 0&quot;</td>
<td>low</td>
<td>-5.16</td>
<td>-4.68</td>
<td>-5.64</td>
<td>4</td>
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<td>8-16-85</td>
<td>4</td>
<td>9' 6&quot;</td>
<td>low</td>
<td>-5.21</td>
<td>-4.53</td>
<td>-5.89</td>
<td>5</td>
</tr>
<tr>
<td>8-16-85</td>
<td>5</td>
<td>9' 6&quot;</td>
<td>low</td>
<td>-5.52</td>
<td>-5.08</td>
<td>-5.96</td>
<td>4</td>
</tr>
</tbody>
</table>

---

**a**. These plants had been submerged by the incoming tide for approximately 1 hour when these samples were taken.

**b**. These plants were the large, sparsely occurring ones in the channel at site 3. The soil samples which follow this entry were taken with the plant samples.

**c**. These plants were the ones occurring in the *Batis* clump in the channel. Again, the soil samples which follow were taken with the plant samples.

**d**. These plants were the very small ones which grew on the bank at site 3. The soil samples which follow this entry were also taken with the plant samples.

**e**. These plants were the same large, sparsely occurring ones which grew in the channel at site 3 and had been sampled previously at high tide.
Fig. 21. Site 1. This site is nearly a pure stand of *S. bigelovii*. The pole used for the site elevation determination reached 8 feet above the soil surface. During a moderate high tide, the pole would be almost entirely submerged. Site 3 is in the background on the left.
These channel plants were growing in a loamy sand (Table 5) that occurred in a slight crown, approximately 100 feet by 200 feet. The first of these channel phenotypes was the very large and bushy, sparsely occurring individuals (Fig. 22). Very few of these plants had neighbors closer than several feet. What was remarkable about these plants is they were huge as compared to the plants at all the other sites. These individuals appeared more succulent and had several times the standing biomass compared to the typical individual in the estuary. The plant heights were about the same in both types, the difference being these large plants had retained virtually all their lower branches.

The second sample type was the plants growing in the same crown only in the midst of a thick clump of *Batis maritima* which was about 15 feet in diameter (Fig. 23). Unlike the large type, these plants were much smaller and more compact with fairly tightly grouped upward pointing inflorescences (Fig. 24). In effect, these were what have been designated as "site 1 type plants." This is the phenotype which overwhelmingly predominates in the estuary.

The third type of individual was discovered on the plateau directly adjacent to and 53 inches higher than the channel in which the other two types occurred (Fig. 25). These plants were quite small, measuring only 4 - 6 inches in height and having 0 to 5 or 6 branches. Numerous of them were a single unbranched shoot.

Site 4 was a shore site approximately 200 yards east of site 1 and was populated with the typical phenotype in the estuary.
Table 5. Particle size analysis, soil type and nitrate content of the soil in Estero Morua. $\text{NO}_3^-$ is given in ppm.

<table>
<thead>
<tr>
<th>Site</th>
<th>% Sand</th>
<th>% Silt</th>
<th>% Clay</th>
<th>Soil type</th>
<th>$\text{NO}_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 3 (channel)</td>
<td>82.2</td>
<td>4.4</td>
<td>13.4</td>
<td>Loamy sand</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Site 3 (bank)</td>
<td>20.2</td>
<td>38.4</td>
<td>41.4</td>
<td>Clay</td>
<td>8.25</td>
</tr>
<tr>
<td>Site 5</td>
<td>73.2</td>
<td>13.4</td>
<td>13.4</td>
<td>Loamy sand</td>
<td>N.D.</td>
</tr>
</tbody>
</table>
Fig. 22. The large, sparsely occurring individuals in the channel at site 3. Plants isolated by only 2 or 3 feet were significantly larger than those growing within about a foot or less of any species. Although the estuary was extensively surveyed, no huge individuals such as this were ever found in close association with any other plants.
Fig. 23. The clump of *Batis maritima* in the channel at site 3 in which numerous *S. bigelovii* occurred. The large, sparsely occurring individuals can be seen around the perimeter of the clump. At low tide times such as this, the estuary can be traversed from shore to shore on foot. The soil remains wet.
Fig. 24. Close-up of the *S. bigelovii* occurring in the *B. maritima* clump in the channel at site 3. These plants appeared identical to the "site 1" phenotype which predominates in the estuary. The first 6 to 8 inches of the stem is unbranched and woody. The remaining upper half of the stem bears branches terminating in strongly segmented infloresences.
Fig. 25. S. bigelovii occurring on the high bank at site 3. These plants were only 5 or 6 inches tall. Many of them, such as the one in the extreme foreground, center, were only a single unbranched shoot. Note in the background, the water of a high tide invading the area. When this high bank position gets flooded, the channel plants at the same site are already under 4 1/2 feet of water. Distichlis spicata occurs here with the Salicornia. This is true in many places in the estuary.
Site 5 was the first site in a string of several at which plant water potentials were taken along the northern (landward) shore in the eastern portion of the estuary. At about 400 yard intervals in an easterly direction from site 5 were sites 6, 7 and 8 which were sampled sequentially just after the retreat of a high tide. Site 5 was later resampled at low tide. Although elevational determinations were not made at sites 6, 7 and 8, an eastward look from site 5 suggested little elevational change in the nearly continuous band of shore occupied by *S. bigelovii*.

Examination of the roots of the plants showed the roots to be from 12 to 14 inches long, although most of the root mass occurred within the first 3 inches of soil (Fig. 26). At 12 inches the roots are so fine, as well as sparse, that it is difficult to imagine any significant amount of water import from a greater depth. For site 1 plants, this places the root zone between 8 feet and 9 feet, 2 inches on the tide chart. For plants at the other sites, the root zone would also extend to 12 to 14 inches below the determined soil surface elevation.

The analysis of the rate of tidal movement showed the tide to be coming in at 1.1 vertical inch per minute. This determination was made during the influx of a tide of intermediate magnitude, meaning this value is a moderate one as well. As is intuitively obvious, the rate of tidal exchange varies with the forces which control the heights of the tides. Tidal rates can also easily be determined from the tide chart.
Fig. 26. Gross root morphology of *S. bigelowii* in Estero Morua. There is not only a very limited amount of root, but most of the root occurs in the first few inches of soil. Threadlike roots extend to a depth of 12 to 14 inches. Overall, the soil volume explored by the roots appears relatively small.
Contrary to the greenhouse grown plants, the vegetative tissue of estuary plants has significantly more ash than that of the reproductive tissue (Table 6), at least at this stage of development.
Table 6. Per cent ash of reproductive and vegetative tissue of *S. bigelovii* in Estero Morua. Ash percentages are means. 95% confidence intervals are 2 standard errors above and below the mean. An inflorescence and a vegetative sample was taken from each individual sampled.

<table>
<thead>
<tr>
<th></th>
<th>Inflorescence</th>
<th>Vegetative</th>
</tr>
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<tbody>
<tr>
<td>% ash</td>
<td>42.23</td>
<td>49.04</td>
</tr>
<tr>
<td>Std. dev.</td>
<td>2.16</td>
<td>3.76</td>
</tr>
<tr>
<td>Std. error</td>
<td>0.82</td>
<td>1.42</td>
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<tr>
<td>95% interval</td>
<td>40.59 - 43.87</td>
<td>46.20 - 51.88</td>
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<tr>
<td></td>
<td>n = 7</td>
<td>n = 7</td>
</tr>
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</table>
DISCUSSION

The nearly identical productivity and yield in the four higher salinities is an uncommon result for most halophytes (Storey and Wyn Jones, 1979; Downton, 1982; Riehl and Ungar, 1983; Ownbey and Mahall, 1983; Key et al, 1984; Glenn and O'Leary, 1984; Cooper, 1984; Waisel, 1985; Robinson and Downton, 1985), but is generally in accord with other performance characteristics in the genus Salicornia (Pearcy and Ustin, 1984). Typical of the genus is the determinate, fixed physiological response and timing of life cycle events (Ungar, 1977; Jefferies, 1977; Jefferies, Davy and Rudmik, 1981).

When viewed with respect to the performance data (Figs 5 and 12, Table 1), the water relations data (Fig. 17, Table 2) indicate a relationship between osmotic adjustment and the ability to attain the standard level of performance. The 10's through 60's each have substantial turgors and virtually identical performances. The turgor differences suggest turgor alone is not the sole determinant of plant growth rates. The common performance in the four higher salinities also indicates osmotic adjustment is not a problem and implies salinity is not a stress as long as drought is not a consideration. The narrow scope of inorganic and organic osmotica in each plant part between the salinities means the plants make small adjustments in ion content, all within a fairly limited range, in order to osmotically adjust. These osmotica contents, the water relations data and the
identical performance at the four higher salinities support the contention that providing sufficient inorganic osmotica is available in the medium, a highly determinate growth response is characteristic of the genus.

The plasmolysis data are revealing (Fig. 28, Table 3). Plasmolysis is fundamentally a binary phenomenon. Either a cell is plasmolyzed or it isn't. The observation that at a particular incubation osmotic potential some cells in the tissue are plasmolyzed while others are not can only mean the tissue is composed of cells having a range of different individual osmotic potentials. In other words, the tissue is really a mosaic of osmotic potentials. This suggests cell environment, such as stomatal or xylem proximity, plays an important role in the determination of the water relations of individual cells. It may be worthwhile to consider plant tissue to be more a population of individual cells rather than a homogeneous tissue. Additionally, the declining slopes of the plasmolysis regression equations indicate at lower cell osmotic potentials, there is a greater range of osmotic potentials among the population of cells. Conceivably, this could buffer the cells against changes in environmental water potential.

In virtually every respect, the 1's behaved differently from all the other treatments (Figs 5, 12, 13, 14, 17, and Tables 1 and 2). However, all treatments establish a substantial water potential gradient, although that of the 1's is somewhat less than the regression equation would predict (Fig. 17, Table 2). Hence, from this standpoint, the 1's appear as functional as the rest and their
deficient performance is not for the lack of a suitable gradient. Interestingly, in spite of the low inorganic ion content of the medium, the l's have nearly the ash content which is predicted by the regression equations (Fig. 13). This indicates they are effective Na\(^+\) and Cl\(^-\) gatherers, but more importantly, it means the plant's ability to supply new cells with the appropriate complement of inorganic ions is a factor dictating the pace of growth. Similarly, Mason and Matsuda (1985) have shown the ability to synthesize protein is as important a growth prerequisite as the threshold turgor claimed by Hsaio et al (1976). In this euhalophyte, ion storage also appears to be critical. Loading the cell with the standard level of inorganic ions appropriate for a given salinity takes precedence over the production of new cells. Considering the dark green, far less succulent appearance of the l's, it may be that relatively high inorganic ion contents are required in the cells for expansion to occur. The extraordinarily high glycine-betaine content of the inflorescence tissue of the l's (Fig. 14) appears to be an attempt to compensate for the inability to supply the new cells in a timely manner with inorganic osmotica from the medium. The inability of the plants to operate at low salinity is further emphasized by their complete inability to reproduce at 0 ppt. In spite of numerous attempts to cultivate the plants to term at 0 ppt, no plant grown without NaCl ever survived long enough to set seed. In early experiments in this research, 0 ppt was typically included as a treatment. While seeds germinated more quickly at 0, within two weeks the seedlings in non-saline treatments developed a characteristic
withered appearance. Individuals were compact, dark green and unable to fully erect themselves. The plants grew very little and rarely survived longer than two months. At the same time, under identical growing conditions, saline treatments of at least 5 ppt were robust, bright green and fully erect. In one experiment, more than 10,000 seeds were sown in a non-saline treatment. Yet in spite of extensive germination, no plant survived long enough to reproduce. Inasmuch as extinction can be defined as the inability to reproduce, this constitutes a sufficient answer to Barbour's (1970) question as to whether there exists an Angiosperm which is truly an obligate halophyte.

The highly standardized performance is consistent with the environmental data from the estuary (Table 4), which suggests that at least as regards water relations, an important determinant of performance, this species occupies a highly predictable niche. Orians (1975) describes salt marshes as having cyclical stability, where the salinity and water potential of the soil fluctuates around the value for seawater. Odum (1969) indicates salt marshes are pulse stabilized systems. It is readily apparent from the tide chart that the plants occur in that portion of the tidal range which receives very frequent and predictable inundations. In fact, there are only 62 high tides out of 730 per year which fall short of the typical root tip elevation of 8 feet. This means the roots experience 91.5% of the high tides during the course of the year. The remaining 62 high tides fall only marginally short of reaching the roots. In addition to the natural watering regime, the nature of the soil largely ensures the plants
never see dryness. After a period of maximum emergence due to a very low tide, a 12 inch deep hole dug for soil water potential samples filled in with water at the bottom almost immediately. Additionally, the extremely fast rate of tidal influx must contribute to oxygenating the incoming water and subsequently lessening anoxia in the sediments. The northern Gulf of California has some of the most extreme tides in the world with a range exceeding 23 feet at Puerto Penasco (Tide Calendar for the Northern Gulf of California, 1985). It is not uncommon to see whitecaps in the estuary, especially if there is an offshore breeze.

While the soil water potential data are inconsistent, there does appear to be a trend towards more negative values at shallower depths (Table 4). This can be ascribed to the synergistic effects of evapotranspiration and percolation of water initially having the osmotic potential of seawater. The result is the soil water potential oscillates between the -2.3 MPa or so value for seawater and somewhere between -4.0 to -4.2 MPa. While the plants never see dryness, most of the roots do experience a water potential cycle which fluctuates with the tides. The very small amount of root tissue (Fig. 26), which appears to be just enough to anchor the plant into the soil, attests to the constant availability of water. Riehl and Ungar (1982) report most of the roots occur in the upper 10 cm of soil in S. europaea, an inland ecotype occurring in a wet, highly saline site. The very similar small amount of roots in inland and coastal species may indicate the extreme salinity tolerance is related to constant water availability. Undoubtedly, the aphyllous habit which minimizes the
transpirational surface contributes to a favorable water relations position. In the estuary, the plants need only to bridge the dip in soil water potential as it falls to values in the high 4.0's during low tides. Beyond this, they appear to have an unlimited supply of seawater.

While these observations make the wide salinity tolerance understandable, they do little to explain the lack of variability in the performance data (Table 1, Figs. 5 and 12). While many halophytes are tolerant of salinities equal to or greater than that of seawater, there is a widely discernable pattern of growth compromise as the price of tolerance. That is, even for halophytes, high salinity is generally a stress. Miohalophytes typically display a linearly declining performance as the salinity of the rhizosphere increases from 0 to higher salinities, while euhalophytes generally have an optima near 10 ppt with growth and yield declining precipitously with decreasing salinities and more gradually at higher ones (Waisel, 1972). O'Leary (1979) has estimated the substantial productivity losses associated with the respiratory expenses of operating at high salinity. Also, Odum (1974) indicates the profound increase in productivity which occurs as a result of the energy subsidy provided by the tides. Hence, the common performance of the four higher salinities in terms of dry weight production and seed yield distinguishes this species from the majority of euhalophytes.

The physiological means by which osmotic adjustment to such a wide range of salinities is accomplished at almost no compromise in productivity and yield has two possible explanations. One would be
for the plant to have an adjustable level of gross photosynthesis such that the higher respiratory costs due to osmotic adjustment are offset by increases in photosynthetic rate. Resources available for growth would be maintained. This confers on the plant the ability to perform better as environmental conditions "decline." Pearcy and Ustin (1984) have demonstrated for *S. virginica*, a California tidal marsh annual occurring in the Sacramento River Delta, that photosynthetic rate is stable over a wide range of salinities. Kuramoto and Brest (1979) also found photosynthesis to be independent of salinity in *S. europaea*, another California salt marsh annual. They also determined respiration to be salinity independent. Inasmuch as extreme salinity tolerance is the trademark of the genus, alterations in photosynthetic and respiration rates are also probably not the basis of the standard performance evident in *S. bigelovii* over such a wide range of salinities.

The alternative, and more likely explanation, is that this ecotype can adjust osmotically at a very minor metabolic expense. From the long term evolutionary standpoint this is not so surprising as natural selection exploits predictability, such as that displayed in the extreme by the tides. The ash content varies only moderately over an extremely wide range of cultivation salinities (Fig. 13). This is especially true of the inflorescence tissue. Once again, the stamp of the genus appears in a relatively fixed, determinate response. The mechanism by which the plants osmotically adjust without measurable effects on growth and yield is related to this narrow scope of osmoticum content inasmuch as the inorganic ion
content of the vacuole accounts for an overwhelming proportion of the osmotica in the tissue.

The different levels of inorganic ions in the three plant parts (Fig. 13) are the result of the developmental pattern of the dicotyledonous herbaceous habit in general and the reproductive format of this species in particular. By the time the plants were sampled at the end of the life cycle, the main stem had become woody and was almost entirely vascular tissue as is typical of an herbaceous dicot stem (Esau, 1977). These conducting systems are non-vacuolated and lack compartmentation. The ash contents represent the inorganic residue remaining in the conducting cells when the tissue has dried, and hence, reflect the osmotic potential of those systems. Several psychrometric measurements made of expressed xylem sap from the pressure bomb indicate the xylem has an osmotic potential of -1.0 to -1.2 MPa (data not shown). Judging from the ash data (Fig. 13), it appears the phloem also has a low to moderate osmotic potential. This indicates the plants are excellent ion entry regulators, as they neither wholly exclude nor freely admit inorganic ions. Considering the high transpiration rates, and the fact that salt glands have yet to be demonstrated in any member of the genus *Salicornia*, the plants would have to be sophisticated ion regulators or the tissue would be entirely salt.

The relatively high inorganic ion content of the inflorescence tissue may reflect the need to maintain a water potential gradient to that tissue after the onset of senescence in the main stem and first few internodes. Near the end of the reproductive period, this species
senesces strongly beginning with the basalmost segments. If, as Weber et al propose (1977), the senescent stem continues to conduct water, and there is no reason to suggest it would not, then the osmotica content would provide for the necessary water potential gradient during seed filling. The time comes, however, when an excised inflorescence will continue to fill seed, indicating that at some point the inflorescence becomes independent of the remainder of the senescing individual. The reverse ash relationship seen in the vegetative and reproductive estuary tissue (Table 6) as opposed to the greenhouse grown samples (Fig. 13) may be indicative of the movement of inorganic osmotica into developing inflorescence tissue. The samples collected in the estuary were from plants developing reproductive tissue while the greenhouse material was post-reproductive when collected and analyzed. Additionally, the large ash percentage of the inflorescence tissue may be to balance the glycine-betaine content of that tissue, which is also substantial (Fig. 14).

Circumstantial evidence suggests glycine-betaine is mobilized during reproduction and translocated to the inflorescence for ultimate incorporation into the seed, as well as possibly for a source of seed protein nitrogen. Salt marshes are notoriously low in nitrogen in their sediments (Valiela and Teal, 1974; Pigott, 1969; and Valiela and Teal, 1979). The nitrogen limited nature of growth of *Suaeda maritima* in a salt marsh has been documented by Pigott (1969) and Stewart, Lee and Orebamjo (1972, 1973). The soil data (Table 5) indicate nitrate nitrogen is also in exceedingly short supply in Estero Morua. Yet large quantities of glycine-betaine, a compound which is 12% nitrogen,
appear in the inflorescence during its relatively short term development. It is difficult to reconcile this rate of accumulation with the undetectable levels present in the sediments. The identical glycine-betaine distribution pattern at even elevated levels in the wild plants implies even more strongly that the plants recycle nitrogen internally to supply the needs of the reproductive tissue. Furthermore, the consequence of unfilled seeds is evident in the 20 ppt treatment (Fig. 16) where apparently for the lack of a place to go, the glycine-betaine has piled up and been left behind in the inflorescence. The very similar amounts of glycine-betaine remaining in the branches and main stems between the different treatments indicates the glycine-betaine has been about equally well mobilized to the inflorescences. When viewed with respect to the annual life history where the parental individual has no future, together these data consitute compelling evidence that the plants are simultaneously using glycine-betaine as an organic osmoticum and a nitrogen storage compound during the growing season. Apparently, when a critical element is rare in the environment, it is essential that it be harvested continually. During the reproductive period, the glycine-betaine may be mobilized to the inflorescence for incorporation into the seeds as either glycine-betaine or as a source of protein nitrogen. This may explain the apparent paradox of why a plant would use a compound which has as a major constituent an element as rare in the environment as nitrogen is in salt marshes for such a critical function as osmotic adjustment.
As with the ash data, there is fairly small variability in the branch and main stem contents of glycine-betaine (Fig. 14), the predominant organic osmoticum in the plant (Wyn Jones, 1980). The distribution pattern in the inflorescence tissue, while less clear, is similar, although this plant part definitely has more glycine-betaine than the other two parts. The parallel levels of inorganic and organic osmotica between the three plant parts suggests a correspondence between osmotica which supports the compartmentation theory of tolerance. Overall though, once again, the organic osmoticum displays limited variability between the four highest salinity treatments, while at the same time the osmotic potentials vary widely. Apparently, by whatever mechanism the cells osmotically adjust, it is not by great variations in osmotica content.

The cells could, however, osmotically adjust at a very limited expense if organic osmoticum was minimal and not a metabolic burden and if the transport and compartmentation of organic and inorganic osmotica were also metabolically inexpensive. Inasmuch as most of the cell is vacuole, and most of the osmotica are inorganic, then a large percentage of the cost of osmotic adjustment may be compartmentation. If, as Dainty (1979) suggests, the tonoplast of halophytes may be less permeable to solutes than that of glycophytes, then the compartmentation costs for the overwhelming amount of the osmotica would be relatively small. There's no question the tonoplast can pump ions. It pumped large amounts of NaCl into the vacuole against a substantial gradient. NaCl must come through the plasmalemma in small amounts and be effectively harvested by the tonoplast. This implies
the tonoplast is strongly impermeable to $\text{Na}^+$ and $\text{Cl}^-$ or those ions would leak back into the cytoplasm and the cell would be continually resequestering the same ions back into the vacuole. Given a fairly stable photosynthetic rate, this use of metabolic energy would appear as diminished growth and yield at the higher salinities. The slightly declining means of plant dry weight and seed yield per plant of the 60's may be a measure of the very low permeability of the tonoplast to these inorganic ions. Additionally, inorganic ions are free for the cost of transport. Overall, this concept that highly efficient compartmentation at the cellular level may be central to the extreme salinity tolerance displayed by this species agrees well with the widely accepted judgement that, in halophytes, large amounts of NaCl are sequestered in the vacuole (Hellebust, 1976; Flowers et al, 1977) and that, at least as regards *S. bigelovii* from Estero Morua, operating at high salinities has little impact on performance.

The data collected in the estuary indicates the plants track the cyclical water potential of the soil (Table 4). Plant water potentials sampled at different times and in different portions of the estuary show a consistent depression below the soil water potential of about 1.5 MPa. In other words, in addition to having a wide salinity tolerance, plant water potentials fluctuate on a tidal basis. This is analogous to the way in which herbaceous terrestrial plants experience a water deficit during the day and rehydrate at night, only in this case on a tidal rather than a diurnal/nocturnal basis. Considering the tidal exchange is a powerful force in the lives of the organisms of the intertidal zone, and is well established as a dominant
evolutionary factor (Brusca, 1980), a mechanism for adjusting to the cyclical water status of the rhizosphere at a very limited metabolic expense is not unlikely. The economy and/or efficiency bases of many adaptive characters in both plants and animals is a widely recognized phenomenon.

Certainly, the tidal regime affects the plant's opportunities to photosynthesize. Evidence indicates S. bigelovii is a C₃ plant. Scanning electron micrographs of cross sections of S. bigelovii from Estero Morua show no indication of the Kranz anatomy (Stumpf and O'Leary, 1984), so this ecotype is not a C₄. Although CAM predominates in succulents, CAM is a water economy format and as such has no place in a frequently flooded coastal estuary. CAM is noticably absent in salt marsh succulents (Winter, 1979). Accordingly, Antlfinger and Dunn (1979) report negligible titratable acidity from the dark to the light period as well as carbon isotope ratios typical of the C₃ pathway in S. virginica, a coastal salt marsh species from Sapelo Island, Georgia. This is consistent with reports indicating other members of the genus are also C₃'s (Tiku, 1976; Kuramoto and Brest, 1979). Carolin, Jacobs and Vesk (1982), in their examination of 33 taxa belonging to the tribe Salicornieae found that only the Halosarcia indica group possessed the carbon isotope ratio and Kranz anatomy characteristic of the C₄ photosynthetic pathway. Finally, Winter (1979) reports "All members of the genus Salicornia thus far examined are C₃ plants." (p. 310).

What could be the nature of the mechanism by which plant water potential tracks just below that of the soil? This question requires
consideration of the relationship between whole plant function on a daily basis and the tidal cycle. If, after all, the tides do dominate the plant's water relations, then events at the cellular level must ultimately relate to the tidal cycle. An examination of the tide chart for a typical month, July, 1985, reveals that the plants spend nearly 33% of the daylight time (6:00 AM to 8:00 PM) submerged. This means the plants experience a restricted photosynthetic period during the day, and may need to fix carbon at a high rate when environmental conditions allow, i.e., during emergent daylight times. Inasmuch as plants basically pay for carbon dioxide with water, and the S. bigelovii in Estero Morua never see drought, it would seem that stomatal conductances would be large and transpiration rates potentially high. This seems a reasonable enough supposition for a plant unfamiliar with drought yet experiencing a significant CO₂ harvesting limitation. Antlfinger and Dunn (1979) have measured high conductances, generally greater than 0.32 cm s⁻¹, in S. virginica in the field. Pearcy and Ustin (1984) have demonstrated the lack of any significant differences in "leaf" and mesophyll conductances in S. virginica over a wide range of salinities. Additionally, the 4 plants in each replicate of the performance experiment transpired about 2 liters of water daily once the plants became sizable. Finally, experiments in which the plants were allowed to experience brief drought indicated unequivocally the plants have virtually no tolerance of drought. Slight drought conditions cause the loss of entire sections of the plant or the death of the individual. This apparent inability to conserve water, even when it is a life or death
situation, suggests they may not even be genetically equipped to do so. Undoubtedly such complete drought intolerance relates to an evolutionary history of copious water use. In its native habitat, this species probably transpires water very freely. Transpiration would, of course, be moderated by the environmental conditions which determine the gradient for the movement of water into the air. The significance of the transpired volume is that it has the potential for being the cause of the observed depression of water potential at low tide.

There are only two ways for tissue water potential to change. The first way would be by the addition of new osmotica which would decrease the osmotic potential of the cells and correspondingly depress the water potential. The literature is replete with reports of osmotic adjustment phenomena based on solute synthesis throughout the plant kingdom and it requires little additional description here. The second way would be for the volume of the cell to change in which case the loss of turgor largely determines the change in water potential while a smaller portion of the change is due to the decrease in osmotic potential which results from the decrease in osmotic volume. Dainty (1979) has indicated that most of the change in cell water potential occurs as a result of the loss of some turgor as opposed to changes in osmotic potential. Based on the available data, it is not possible to say to what degree turgor would be altered with a given loss of water from the cell. Plant cell walls have an elastic quality to them which influences the change in turgor as cell volume changes (Dainty, 1979). Cells which have a lower elasticity modulus
are more elastic and experience smaller turgor variations with a given loss of water than cells of a less elastic nature. Also, the elasticity modulus of a cell is not a constant, but varies with cell volume. Sufficiently elastic walls would allow the cell to maintain a substantial turgor in the face of a modestly declining cell volume and water potential. Of course, the change in water potential may be a combination of both solute synthesis and a change in cell volume.

The problem with the first approach is that it is contrary to an established tenet of evolutionary theory that natural selection exploits predictability. For a tidal plant to undergo a never ending solute synthesis and degradation program puts the plant in the position of responding to the changing soil water potential as if the species had no genetic awareness of the cycle. The Gulf of California is about 5 million years old (van Andel, 1985), and it is not possible to say how long S. bigelovii has been out there. Yet considering the range of adaptations required to survive as an annual in this coastal estuary, as well as the metabolic inefficiency involved, this approach is clearly untenable. Hence, the change in plant water potential must relate overwhelmingly to a change in osmotic volume most likely due to transpiration at a time when the soil water potential is declining. The transpired volume may simply exceed water import through the roots at low tide and the cells experience a slight draw as the water potential of the transpirational stream declines below their own. This is not to say the cells provide most of the transpired volume. Unquestionably they don't. Most of the transpired water moves through the plant and out the stomates without interacting with the tissue as...
a whole. It does mean, however, that there is a water potential gradient from the parenchyma to the xylem and/or apoplasm and that the cells apparently contribute enough water to the transpirational stream to depress their water potential through the previously described change in turgor and osmotic potential.

While the volume of the cell may change within certain latitudes, it is not an event without consequence. Retention of the contents of the cell is a plasmalemma function and therefore the non-aqueous constituents in the cytoplasm and vacuole remain when water is lost from the cell. The unavoidable result is an increase in the concentration of the soluble components of the cell. It appears, however, that protein synthesis is very sensitive to the concentrations of K+ and Mg2+ (Roberts and Paterson, 1973). This can only mean that for metabolism to function, the volume of the cytoplasm must be maintained within a specific range. This does not necessarily mean the volume of the cell may not change more radically. But how can the volume of the cell change substantially while that of the cytoplasm remains within much narrower limits?

Plant cells are approximately 80 – 90% vacuole. Inasmuch as the vacuole is typically assigned a cytoplasmic storage and supply function, the disproportionate volumes of the two compartments would seem to be some form of risk/safety assessment on the part of natural selection much in the way that plants manage their root/shoot ratios. The increase in root/shoot ratio with drought stress is a well known phenomenon. This is not a bad idea for organisms which must face the environment fixed in place for the life of the individual. If indeed
the vacuole is a storage compartment for the cytoplasm, then it might be the means by which the cytoplasm maintains an appropriate volume in the face of an overall declining cell water content.

This shift in the relative volumes of the two compartments could be accomplished by a shuttling mechanism which transported compatible organic osmotica from the vacuole to the cytoplasm. While it is widely accepted that for enzymatic reasons the inorganic ions are largely sequestered in the vacuole (Flowers et al, 1977), the organic osmotica can be in either compartment. The crucial consideration here is that the cytoplasm is only on the order of 10 - 20% of the volume of the vacuole, and therefore the movement of an ion pair across the tonoplast and into the cytoplasm causes a much greater decrease in the osmotic potential of the cytoplasm than the corresponding increase in that of the vacuole. Water potentials would be displaced accordingly. The result would be a net flow of water from the vacuole to the cytoplasm, compensating the cytoplasm for its modest loss of water to the transpirational stream. In effect the vacuole would act as a reservoir for the cytoplasm at compartmentation and transport expense. Overall, the water potential of the cell would decline, as the data show, but the volume of the cytoplasm, and therefore the metabolic environment, would be fairly stable. McNulty (1985), in his experiments involving osmotic adjustment in *S. europaea*, has concluded that "As there was no significant increase in any compatible osmotica, the osmotic stabilization of the cytoplasm must have been largely a reapportion of compatible osmotica within the cell, e.g. transfer from the vacuole to the cytoplasm." (p. 103).
Sinclair and Ludlow (1985) have very recently pointed out the extensive inadequacy of thermodynamic variables as measures of physiological performance. Additionally, they state, "There is increasing evidence to support the view that cell volume, or more commonly relative water content (RWC), is an important, and possibly major determinant of metabolic activity and leaf survival." (p. 214). However, it may not be the RWC of the cell per se which is important, but the maintenance of a cytoplasmic volume which corresponds to metabolically conducive concentrations of cytoplasmic constituents. For a coastal halophyte such as *S. bigelovii* which experiences frequent and predictable inundations, such a mechanism would allow the plant to maintain high stomatal conductances and CO₂ harvesting capabilities, while simultaneously providing a metabolic environment for competent CO₂ fixation and subsequent metabolic activity. This is, of course, the low tide portion of the cycle. During high tides when the soil was saturated at a water potential that was that of seawater, the cells would have to rehydrate and the glycine-betaine would be resequestered in the vacuole thus restoring the higher water potential associated with a greater RWC in preparation for the next cycle. It must be borne in mind that while newly formed cells in the meristematic region undoubtedly require a threshold turgor for expansion, fully expanded cells which comprise probably 99% of the plant may have the maintenance of a proper osmotic volume of the cytoplasm as the critical and ultimate water relations criteria.
CONCLUSIONS

1. When grown in a range of salinities from 10 to 60 ppt, *S. bigelovii* has a standard growth and seed yield. Plants grown at low salinity, such as 1 ppt, have less than half the productivity of higher salinity ones.

2. The plants are able to generate substantial water import gradients, water potentials, osmotic potentials and turgors over the wide salinity range of 10 to 60 ppt. At 1 ppt, there are large import gradients, but turgor is low. Overall, 1 ppt treatments appear to be outside the wide range of salinities capable of producing the standard growth and yield responses. This may indicate the ability to accumulate ions in a timely manner is an important growth prerequisite.

3. Inorganic and organic osmotica levels increase linearly but slightly in inflorescence, branch and main stem. One ppt inflorescence tissue has exceptionally high levels of glycine-betaine. These data support the compartmentation theory of salinity tolerance in halophytic Angiosperms.

4. In spite of numerous attempts to cultivate plants to term at 0 ppt, no plant in an unsalinized treatment ever produced seed.
Inasmuch as the inability to reproduce leads absolutely to extinction, this plant must be an obligate halophyte.

5. Throughout the northern shore of the estuary, the species occupies a discrete elevational range in which the plants experience approximately 92% of the high tides. This position and the water retention of the soil ensure the plants never see dryness.

6. Meristem water potentials fluctuate with the tides, remaining about 1.5 MPa below the corresponding soil water potential values. The highly predictable salinity associated with the tidal cycle may be the basis of the determinate growth and yield responses.

7. The plants appear to have just enough root to anchor them into the soil. This attests to their unfamiliarity with drought.

8. In this species, nitrogen metabolism and water relations may be intricately related as the plants appear to use glycine-betaine for simultaneous osmoticum and nitrogen storage.

9. It is suggested that the extreme salinity tolerance of this species is based on highly efficient compartmentation at the cellular level. Additionally, it is hypothesized that the shuttling of the organic osmoticum glycine-betaine is the basis of the cyclical water potentials of estuary plants.
MODIFIED HOAGLAND'S SOLUTION

The composition of the nutrient solution was as follows:

Macro/micro:
1 mole of \(\text{KNO}_3\)
1 mole of \(\text{KH}_2\text{PO}_4\)
1 mole of \(\text{MgSO}_4\)
0.75g of \(\text{MnCl}_2\)
1.25g of \(\text{H}_3\text{PO}_4\)
0.025g of \(\text{CuCl}_2\)
0.21g of \(\text{MoO}_3\)
0.19g of \(\text{ZnSO}_4\) all dissolved in 3.5 liters of distilled water.

Additionally, a 1 molar solution of \(\text{Ca(NO}_3)_2\) was prepared.

To prepare the final nutrient solution, 175 ml of the macro/micro and 75 ml of the \(\text{Ca(NO}_3)_2\) solution were added to 25 liters of distilled water. Then, 1.3 g iron chelate (Sequestrene) was added to this 25 liters to give a final concentration of 5 ppm. The solutions were then salinized to the proper level for each treatment.
APPENDIX B

PLASMOLYSIS PROCEDURE

First, a graded series of sucrose solutions was prepared at 0.5 MPa intervals having a range which was believed to include the actual osmotic potential of the cells. Published tables are available such as that of Molz (1926) which list molar concentrations of sucrose and their respective osmotic potentials. In order to estimate the cell osmotic potential, cell water potential was first determined psychrometrically and the assumption was made that turgor would be some value greater than 0 yet less than 1.5 MPa. Consequently, this meant that the absolute value of the true cell osmotic potential would lie in a range which was between 0 and 1.5 MPa greater than the absolute value of the determined water potential. In each case it was attempted to select a range which would include the actual cell osmotic potential at approximately midway in that range. In effect, when the percent of plasmolyzed cells in a particular sucrose solution was plotted against the osmotic potential of that solution, the point of incipient plasmolysis on the regression line would turn out to be somewhere near midway in the range of osmotic potential selected for that particular analysis. The osmotic potential of the cells is taken to be equal to the osmotic potential of the incubation solution which according to the regression line causes plasmolysis in 50% of the cells (Barrs, 1968). This is the point of incipient plasmolysis.
Subsequently, six incubation chambers, one for each osmotic potential treatment, were prepared using standard plastic 10.0 cm diameter petri plates. Rubber stoppers approximately 2.0 cm in diameter were sliced to produce discs about 0.5 cm thick to be used as slide pedestals. Next, a Kimwipe was neatly folded and set in the bottom of the petri plate. This Kimwipe was saturated with the sucrose solution having the osmotic potential of that treatment. Then the rubber stopper disc was placed on the saturated Kimwipe and the system was immediately covered with the petri plate lid. It is important to do this fairly quickly so as to minimize evaporation from the sucrose solution and consequent change in its osmotic potential. In this way an incubation chamber was created which would suspend the 1 x 3 inch glass slide bearing the epidermal peels in a closed atmosphere having a water potential equal to the osmotic potential of the incubating solution. Hence, there should be virtually no evaporation from the incubation solution on the slide during the incubation period and the tissue should experience a reasonably constant solution osmotic potential.

Having prepared the sucrose solutions and the incubation chambers, the procedure for quickly making the epidermal peels and mounting them on a glass slide for incubation was as follows:

1. Using a pipetman, 25 microliters (μL) of the first incubation solution was withdrawn from the stock bottle. Approximately one quarter of this volume was dispensed in a small bead on a clean glass slide in the area where the 24 x 50 mm cover slip would eventually be set.
2. Using a fresh single edge razor blade, the last 2 mm of a healthy looking shoot tip was excised and immediately sealed in a psychrometer for immersion in a 24.5 C water bath. The psychrometers were read 4 hours after tissue sampling. Immediately following the loading of the psychrometer, the next 2 mm of the same shoot tip was excised and quickly given a shallow longitudinal slit, leaving the blade in the incision. Using the blade to hold the cylinder of tissue against the thumb, the index finger was used to roll the cylinder thus cleanly peeling the epidermis away from the remainder of the tissue. With practice, this peel could be made in a matter of seconds.

3. Immediately, the epidermal peel was placed cuticular side down on the bead of solution on the slide. This serves to prevent the peel from curling. Peels were viewed and photographed from the inside as opposed to through the cuticle which obscures the view. Following placement of the peel on the slide, just enough fluid from the reservoir in the pipetman was gently dispensed to cover the cells. In this way, tissue dehydration was avoided.

4. As quickly as possible, the previous steps were repeated until there were three well spaced peels on the slide.

5. As soon as the last peel was in position, the remaining solution in the pipetman was carefully distributed over the three peels.

6. The cover slip was then gently placed on the slide, avoiding the entrapment of air bubbles on the tissue. Bubbles prevent effective viewing and photography. The easiest way to set the cover slip on was to place one edge on the slide and slowly lower the other
edge using the razor blade. When it appeared that air bubbles were going to be entrapped around the tissue, the cover slip could be carefully raised and lowered repeatedly with the blade until the bubbles migrated away from the tissue. When no air bubbles were in contact with the peels, the cover slip was let down all the way and released. When the slide is completed and the entire 25 uL is under the cover slip, the slip floats on the solution. A fluid volume greater than 25 uL allows the peels to curl such that they do not present a planar surface for viewing or photography.

7. As soon as a slide was completed, it was placed on the rubber stopper disc in the appropriate incubation chamber and the chamber was positioned out of the way in the dark in the same air-conditioned room. The room temperature was typically 24.5 C. Careful, level placement of the slide on the disc is important so as to prevent the loss of fluid over the edge of the slide.

8. Slides were incubated for 4 hours before being examined and photographed under the microscope.

9. Four hours was empirically determined to be the most reasonable time for equilibrium attainment. This was determined by preparing a slide as just described and photographing it at 15 minute intervals for a 5 hour period. A plot of percent plasmolysis against time produced a sigmoidal curve having three distinct regions (Fig. 27). In the first region, there was relatively slight plasmolysis as time proceeded. This was followed by an area of extensive plasmolysis in a fairly short period. The final phase depicted a very slight increase in the percent plasmolysis with additional time. The point
Fig. 27. Determination of plasmolytic equilibrium. See Appendix B, step 9, for the details of this graph.
on the abscissa where the transition from the second to the third phase occurred was taken as the attainment of equilibrium. The basis for this judgement is that equilibrium could not have occurred prior to the burst of plasmolysis which defines the second phase, yet the third phase most likely depicts the residual plasmolysis still occurring as well as the slight invasion of the tissue by the sucrose which has been occurring all along. However, at this point the invasion component may represent a significant part of the very small change seen in the third phase. Therefore, the breakpoint between these two phases must represent the most reasonable attainment of true equilibrium.

10. At the completion of the equilibrium period, in the order in which they were prepared, the slides were photographed using an American Optical microscope equipped with an AO Expostar Shutter Control and an AO model 1053C 35 mm photomicrographic camera (American Optical Corp., Buffalo, N. Y.) loaded with Kodak 160 ASA Tungsten film (Kodak Corp., Rochester, N. Y.). For each peel, the first randomly selected photographically acceptable field was photographed. The numbered 35 mm slides were randomized by shuffling and then projected on an 8 1/2 x 11 sheet of white paper such that all fully in view, well focused cells fit on the paper. Then those cells were numbered and the plasmolyzed ones marked so as to allow for the determination of the percent of cells plasmolyzed. This was done for each of the three peels in a treatment and for all six treatments. Hence, each regression line was determined by eighteen points, each representing the percent plasmolysis of an individual epidermal peel. In four
instances, the entire peel was sufficiently out of focus that it could not be analyzed.

11. Finally, the percent plasmolysis was plotted against the osmotic potential of the incubation solution to yield Fig. 28.
Fig. 28. Percent plasmolysis as a function of incubation osmotic potential. The procedure by which the data for this graph was obtained is detailed in Appendix B.
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