

SPATIAL AND TEMPORAL VARIATION IN TISSUE HALIDE LEVELS OF *SALICORNIA VIRGINICA*

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Abstract: Succulence, tissue chloride, bromide, and iodide levels were determined in the coastal salt marsh halophyte *Salicornia virginica* taken from three elevations over a 12-month period. Soil samples from each sampling area were also taken for the determination of halide concentrations. The average percent dry weight (% DWt) over the 12 months and three elevations ranged from 10.8 ± 0.4 to 16.1 ± 0.3 % (\pm sd), with a general increase in % DWt (decrease in succulence) in tissue from all elevations peaking in September, after the dry summer, followed by a decrease in % DWt (increase in succulence) during the rainy season. The mean halide levels in *S. virginica* tissues over the 12 months and three elevations ranged from 17.5 to 30.3 % Cl^- , 0.27 to 0.76 % Br^- , and from 3.00 to 1.21×10^4 ppm I^- . Mean halide concentrations in plant water ranged from 0.66 to 1.6 M Cl^- , 4.6 to 12 mM Br^- and 3.3 μM to 16 mM I^- . No environmental factors correlated with changes in tissue halide levels. Tissue Cl^- levels tended to increase during the dry summer months and decrease during the wet winter months. Bromide levels were more stable. Large increases in tissue I^- levels were found during the months of September and October when the plants were in flower, suggesting a developmental control of I^- uptake. Soil concentrations of all three halides at the low elevation were consistently higher than those in the middle and high elevations. The high elevation soil Cl^- was the most variable of the three halides. Higher I^- soil levels in the low elevation during the spring/summer as compared to fall/winter probably reflect an increase in the biological and chemical reduction of IO_3^- to I^- . Tissue halide levels at the three elevations did not correlate with their respective soil concentrations. Plant water halide molar ratios were lower than in seawater and soil, indicating the selective uptake of halides in the order $\text{I}^- > \text{Br}^- > \text{Cl}^-$.

Key Words: *Salicornia*, halide, iodide, halophyte, coastal salt marsh

INTRODUCTION

Halophytic plants of the genus *Salicornia* are succulent, shallow-rooted, and have a wide distribution in coastal salt marshes (Zedler 1982). There are three species of *Salicornia* found in southern California salt marshes: *S. virginica* L., *S. bigelovii* Torr., and *S. subterminalis* Parish. *Salicornia virginica* is a perennial that forms dense stands from Baja California to Washington State (Boyer et al. 2001). *Salicornia virginica* also has the widest distribution in coastal salt marshes, being found in all marsh elevations. It is most abundant, however, in the lower and middle marsh elevations (Zedler 1982). *Salicornia virginica* is suffrutescent, with succulent stems and small scale-like leaves (Purer 1942). The succulent green stems may turn red with age prior to becoming woody.

Coastal salt marshes are extremely productive, have high species diversity, and have large plant biomass. They are characterized by steep gradients of salinity, oxygen concentrations, and soil moisture. The major primary producers are halophytic plants, such as *Sal-*

icornia, whose zonation is mainly defined by horizontal gradients, salinity being particularly important. Tidal flow and freshwater input (rivers and rainfall) cause salinity to fluctuate daily and seasonally, while other less transient factors, including soil texture, vegetation, and water-table depth, can also influence soil salinity (Mitsch and Gosselink 2000).

Coastal salt marshes and estuaries are also sites of extensive biogeochemical transformations of nutrient and non-nutrient elements. Some of these transformations involve the methylation of metals (i.e., Sn, Hg, Se) and halides (Ring and Weber 1988, Weber et al. 1998, Tessier et al. 2002). Methyl halides (monohalomethanes) are chemically important atmospheric constituents and significant vectors of halogen transport from terrestrial and oceanic ecosystems to the atmosphere (Wofsey et al. 1975). Because of their relatively long residence times, CH_3Cl and CH_3Br can diffusively reach the lower stratosphere where they (i.e., halogen atoms produced from their photodissociation) catalytically destroy the ozone layer. Despite

its short residence time, CH_3I has recently been shown to be injected into the lower stratosphere by large convective events where it can react with stratospheric ozone (Solomon et al. 1994). As a major source of iodine atoms in the troposphere, CH_3I and other iodomethanes strongly influence tropospheric chemistry, participating in ozone depletion, HO_2/OH modulation (Davis et al. 1996, Carpenter et al. 1999, Vogt et al. 1999) and supplying “condensable iodine vapor,” which promotes the formation of marine aerosol and cloud condensation nuclei (O’Dowd et al. 2002). Coastal salt marshes are estimated to produce approximately 10% of the total global flux of CH_3Cl and CH_3Br to the atmosphere based on measurements from southern California locales. Methyl chloride and CH_3Br emissions were highest in the middle to upper-middle intertidal area where the halophytes *Batis* sp., *Frankenia* sp., and *Salicornia* sp. dominate (Rhew et al. 2000).

Although the importance of coastal salt marsh methyl halide production is known, little is known about the halide content of the plants that produce them. To our knowledge, halophytic plant tissue levels for chlorine, bromine, and iodine (presumptive for respective halides) have been published only for *Rhizophora mangle* (L.) (red mangrove) and *Aster tripolium* (L.) (sea aster; Market and Jayasekera 1987). Chloride content has been reported for several halophytes including *S. europaea* (L.) (Keiffer and Unger 1997) and *S. herbacea* (L.) (Chapman 1942). The purpose of this study was to determine the level of tissue halides (Cl^- , Br^- , and I^-) in a population of *S. virginica* and to determine if these halide levels vary with season, tidal flux, and elevation. We chose *S. virginica* for this study because it has a wide distribution within the marsh habitat, stores halides in vacuoles, can tolerate a large range of salinities, and emits methyl halides.

MATERIALS AND METHODS

Field Sampling of Plant Tissue and Soil

Samples were collected from Newport Back Bay, California, near the old salt dike ($33^\circ 38.75\text{N}$, $117^\circ 52.94\text{W}$) (Figure 1). *Salicornia virginica* tissue was collected monthly for a period of 12 months starting on 2 April 2002 and ending on 1 March 2003. Samples were taken from three areas that were separated by 2 m and at elevations that differed in relation to the mean sea level (MSL). The low elevation sampling area was 1.03 m above MSL. The middle sampling area was located on a slight elevational rise, with the lowest corner of the quadrat 1.19 m and the highest corner 1.76 m above MSL. The high sampling area was located behind a small concrete-wall remnant of

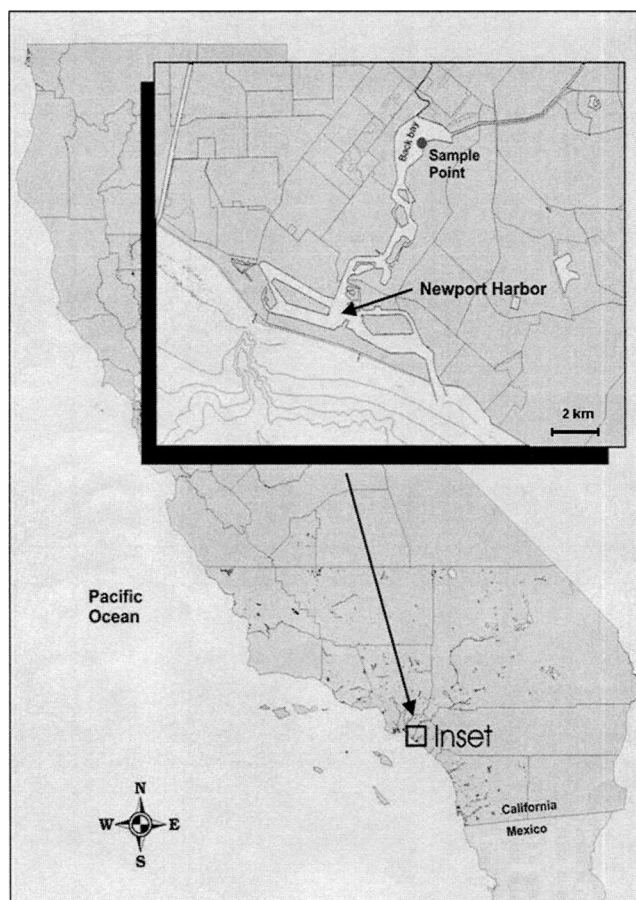


Figure 1. Map of the sampling point located in Newport Back Bay, California, USA ($33^\circ 38.75\text{N}$, $117^\circ 52.94\text{W}$).

an old salt dike, 2.04 m above MSL. The low area was inundated daily by the tidal flow, the middle area was only inundated on spring tides, and the high area did not receive any tidal flow.

A completely randomized experimental design was used to select samples. A $2 \times 3 \text{ m}^2$ sampling PVC pipe/rope quadrat with 0.25 m^2 grids within the quadrat, resulting in 96 squares, was used. With the quadrat laid out, the right corner was marked with a stake to ensure placement in the same area each sampling period. A random numbers table was used to select six different squares from which tissue was collected. Approximately 10 g fresh weight of *S. virginica* tissue (1–3 plants, no effort was made to take tissue from separate plants) from each randomly selected square, for a total of 60 g of tissue from each sampling area. Only the upper 50 mm of green tissue was taken because this was the youngest tissue, which was presumed to contain the least amount of halides and be the most susceptible to variations in halide concentration in soil pore water. Red-tipped older tissue and flowering tissue was avoided to ensure that samples would be as similar as possible. Two soil samples (50

Table 1. Accuracy and precision of halide analysis.

Halide	Mean Standard Check (M)	Accuracy (%)	Standard Deviation (M)	Precision (% sd)	Standard (M)
Chloride ($n = 51$)	1.02×10^{-3}	98%	5.84×10^{-5}	5.7%	1.0×10^{-3}
Bromide ($n = 89$)	5.58×10^{-5}	88%	6.21×10^{-6}	11%	5.0×10^{-5}
Iodide ($n = 121$)	1.05×10^{-6}	95%	8.43×10^{-8}	8.0%	1.0×10^{-6}

g taken from the east-west side of the quadrat) were taken every 3 months (April, July, October 2002 and January 2003) at each elevation. All samples were stored in plastic resealable bags on ice for return to the lab.

Plant Tissue and Soil Halide Extraction and Analysis

After the fresh weights of the tissue samples were determined, they were rinsed in deionized water to remove any external salts. The tissue was dried to constant weight at 55° C in an electric gravity convection oven (Precision Model 25EG) for approximately 72 hours. After drying, the tissue was weighed on a Mettler PB602S balance, milled into a fine powder on a Wiley milling machine (40- μ m mesh), and stored in a capped glass vial under desiccation. Average solid content of tissue (% DWt) was calculated for each month and elevation from tissue wet and dry weights. Soils were dried to constant weight at 40° C, passed through a 2-mm sieve, and stored at room temperature.

Halides were extracted from the milled plant tissue by adding the powder (0.5 g) to 50 ml of boiling deionized water in a 150-ml flask. This mixture was boiled for 10 minutes then vacuum-filtered through filter paper (Whatman # 2). The volume of filtrate was measured and stored frozen until halide analysis. The extraction procedure was repeated five times on the same sample to ensure complete halide removal from the tissue. Greater than 98% Cl^- , 97% Br^- , and 99% I^- was removed from the tissue after three sequential extractions. Halides were extracted from the dried soil samples by washing the soil (25 g) in 50 ml of deionized water in a 150-ml flask. The mixture was placed on a shaker (200 rpm) for one hour, after which it was vacuum filtered through filter paper (Whatman #3). This procedure was repeated three times to ensure that all extractable halides were removed from the soil. The volume of filtrate was determined and stored frozen until halide analyses were performed.

The halide concentration in each extract was determined using a specific ion meter/autotitrator in conjunction with the appropriate halide electrode by the Gran's known-addition method (Gran 1952, Redeker et al. 2002). This method allowed for the measurement of halides near their limit of detection: 5×10^{-5} M

Cl^- ; 5×10^{-6} M Br^- ; and 5×10^{-8} M I^- (Thermo Orion specifications). Following the auto-titration, the calculated initial concentration (in mM) was reported. The mean standard check for accuracy (with precision) of the analysis ranged from 98% Cl^- to 88% for Br^- (Table 1). Halide concentrations in mM were converted to grams halide per grams dry weight of tissue, expressed as either % (i.e. % DWt) for Cl^- and Br^- , or ppm for I^- . The concentrations of halides in the tissue water were calculated based on the amount of water removed from the tissue.

Statistical Analyses

To test for significant differences among months and elevations, a two-way (GLM) ANOVA was performed on SAS. The least square means pair-wise comparison was appropriately used to test for differences among each group mean. Effects were considered significant using $\alpha = 0.05$ (one-tailed). In order to meet the assumptions of ANOVA, the data for I^- were \log_{10} -transformed prior to the statistical analyses; although Cl^- and Br^- were not transformed, data were not pooled for analyses. Correlation analyses were used to look for positive relationships between halide concentrations and environmental factors. These environmental factors included mean water temperature (vs. middle and low areas only), air temperature, monthly rainfall, time since last rainfall, rainfall strength (rainfall amount \div time since last rainfall), bay water salinity, height of last high tide, time since last high tide, tidal strength (height of last high tide \div time since last high tide), tidal height at sampling, and soil halide levels.

RESULTS

The average percent dry weight ranged from 10.8 ± 0.4 to $16.1 \pm 0.3\%$ (\pm sd) over the 12 months and three elevations (Figure 2). The lowest mean percent dry weight of 10.8% was found in January 2003 from the low elevation, and the highest mean of 16.1% was found in September 2002 from the middle elevation. There appears to be a general increase in percent dry weight for tissue from all elevations peaking in September, after the dry summer, followed by a decrease

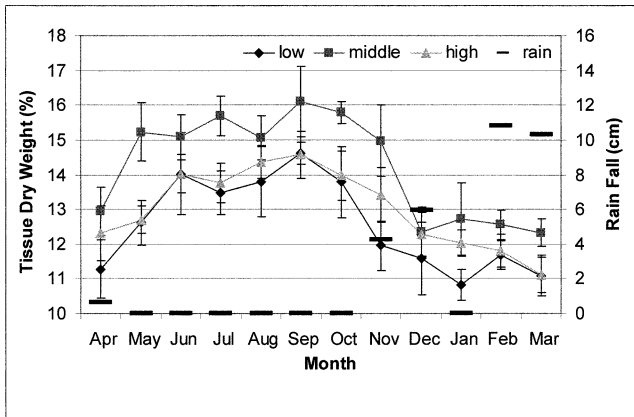


Figure 2. Mean percent dry weight (\pm) standard deviation of *Salicornia virginica* tissue over the 12 sampling months and three elevations in Newport Back Bay and monthly rainfall from nearby Costa Mesa, California for 2003–2004 (Orange County Watershed and Coastal Resources Division 2004).

during the rainy season. The percent dry weight at the middle elevation was greater than the other two elevations each month sampled. The mean Cl^- levels in *Salicornia virginica* tissues ranged from 17.5 ± 1.2 to $30.3 \pm 1.7\%$ (\pm sd) over the 12 months and three elevations (Figure 3a). Significant overall differences were found among months ($F_{11,180} = 39.81$, $p_{\text{month}} < 0.0001$), and elevations ($F_{2,180} = 4.45$, $p_{\text{elevation}} = 0.0129$). A significant interaction was also found for month and elevation on Cl^- content ($F_{22,180} = 2.96$, $p_{\text{interaction}} < 0.0001$). The least square means pairwise comparisons showed that the highest mean (30.3%) found in July 2002 at the low elevation was not significantly greater than any other mean. The lowest mean (17.5%) found in February 2003 at the high sampling area was not significantly lower than any other mean.

Monthly analysis of tissue Cl^- levels showed that there were no significant differences in the mean percent tissue Cl^- from the three different elevations for the spring months of April, May, and June 2002 (Figure 3a). There was an increasing trend in the mean Cl^- levels during May and June as compared to April. The summer months of July, August, and September 2002 were less variable than the spring months. The months and elevations of August and September were not significantly different from each other. However, tissue concentrations in the low and middle elevations during July were significantly higher than the other group means measured during that time period. During the winter months of January, February, and March 2003, there was a general decreasing trend for all three months and elevations as compared to December. Overall, there was a general increase in mean percent

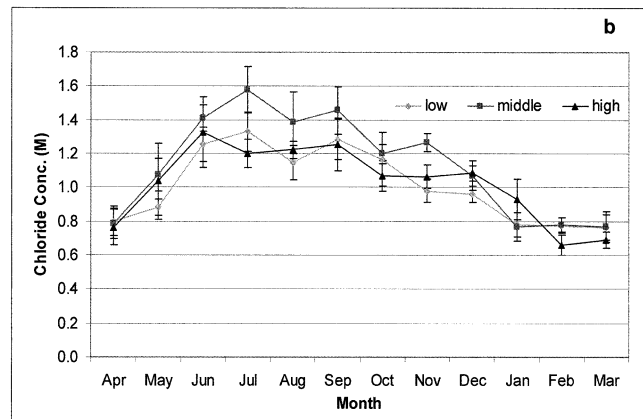
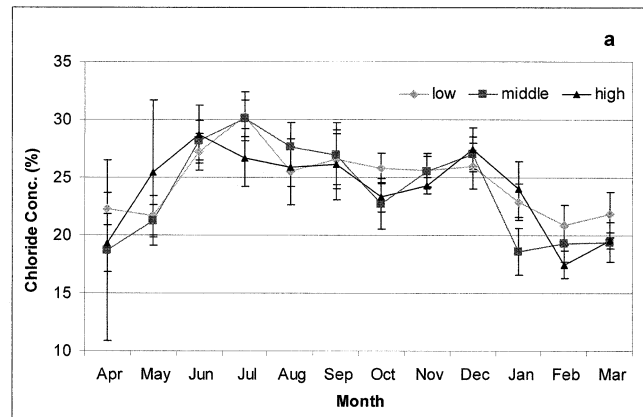


Figure 3. Tissue chloride levels (a, % dwt) and plant water chloride concentrations (b, M) in *Salicornia virginica* tissue over 12 months and three elevations from Newport Back Bay, California. (\pm sd)

Cl^- levels during the dry summer months, followed by a decrease during the wet winter months.

The mean Br^- levels in *Salicornia virginica* tissue ranged from 0.27 ± 0.02 to $0.76 \pm 0.17\%$ (\pm SD) over the 12 months and three elevations (Figure 4a). Significant differences were found among months ($F_{11,180} = 20.08$, $p_{\text{month}} < 0.0001$) although no significant differences were found among elevations ($F_{2,180} = 2.06$, $p_{\text{elevation}} = 0.1303$). The patterns of Br^- levels, over time, were different among the elevations ($F_{22,180} = 11.58$, $p_{\text{interaction}} < 0.0001$). The least square means pairwise comparisons showed that the highest mean (0.76%) found in *Salicornia virginica* tissues in April 2002 from the low elevation was significantly greater than any other mean. The second highest mean of 0.61% found in October 2002 at the high elevation site was also significantly different from all other means. The overall lowest mean Br^- level of 0.27% found in January 2003 at the middle elevation was not significantly lower than any other mean.

Monthly analysis of Br^- showed that the mean tissue levels in April 2002 were highly variable (Figure

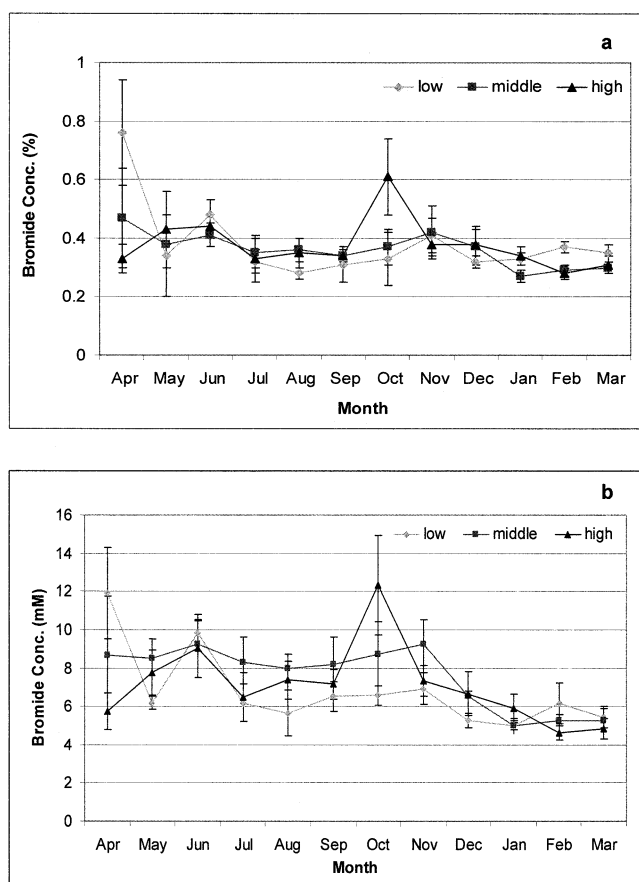


Figure 4. Tissue bromide levels (a, % dwt.) and plant water bromide concentrations (b, mM) in *Salicornia virginica* tissue over 12 months and three elevations from Newport Back Bay, California. (\pm sd)

4a). For April, the mean tissue Br^- levels at all three elevations were significantly different from each other, while in May and June, no significant differences were found among the elevations. Bromide tissue levels in April were also significantly different from May and June, which were not significantly different from each other. During the summer months and across elevations, Br^- tissue levels were not significantly different from each other. This was also true for the winter months of January, February, and March 2003. Further analysis showed that Br^- levels in the winter or summer months were not significantly different from each other. The fall months contained a single high Br^- mean of 0.61%, which was significantly different from all other means. Disregarding this single high value, none of the other means from any elevation or month were significantly different during the fall. Overall, the tissue levels of Br^- were stable after the first sampling month of April 2002.

The mean tissue I^- level ranged from 3.00 ± 1.20 to $1.21 \times 10^4 \pm 3.80 \times 10^3$ ppm (\pm sd) over the 12 months and three elevations (Figure 5a). Significant

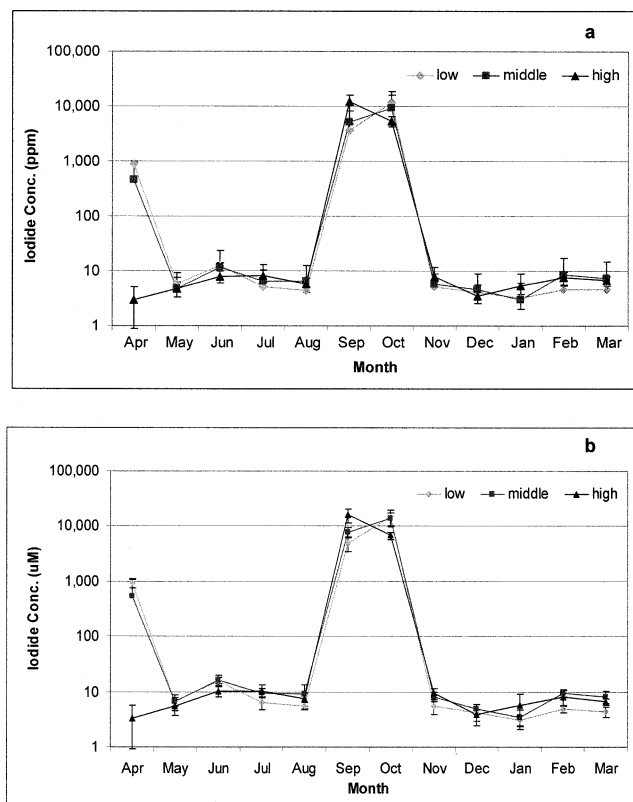


Figure 5. Tissue iodide levels (a, ppm) and plant water iodide concentrations (b, μM) in *Salicornia virginica* tissue over 12 months and three elevations from Newport Back Bay, California. (\pm sd)

overall differences were found among months ($F_{11,180} = 557.21$, $p_{\text{month}} < 0.0001$) and elevations ($F_{2,180} = 8.20$, $p_{\text{elevations}} = 0.0004$), and a significant interaction was found between month and elevation ($F_{22,180} = 20.65$, $p_{\text{interaction}} < 0.0001$). The tissue I^- level of 1.21×10^4 ppm found in September 2002 from the high elevation was significantly greater than the I^- levels in all other samples, except those from October 2002 from both the low and middle elevations. The lowest mean level of 2.98 ppm I^- found in April 2002 in the high elevation was not significantly lower than other mean levels in May through August 2002 or November 2002 through March 2003.

Tissue I^- levels showed large fluctuations during different seasons. As with Br^- , tissue I^- levels measured in April 2002 were highly variable. For the month of April, the tissue levels in both the low and middle elevations were significantly greater than the high elevation. The mean I^- level dropped precipitously for both the low and middle elevations in May. The summer months started with low mean levels for both July and August; however, tissue I^- levels increased dramatically during the month of September. This extremely high I^- level continued through the

Table 2. Mean soil, tissue and tissue water concentrations of halides¹.

elevation	low	sd	mid	sd	high	sd
soil levels (n = 8)						
chloride (% DWt)	4.85	0.77	2.37	1.05	5.77	4.62
bromide (% DWt)	0.054	0.029	0.030	0.014	0.035	0.019
iodide (ppm)	4.29	1.63	0.65	0.27	0.65	0.38
tissue levels (n = 72)						
chloride (% Dwt)	24.7	3.2	23.8	4.4	24.0	4.2
bromide (% Dwt)	0.38	0.14	0.36	0.08	0.38	0.1
² iodide (ppt; ‰)	1.37	3.52	1.25	2.98	1.46	3.70
tissue/soil						
chloride	5.1		10		4.16	
bromide	7.0		12.0		10.9	
iodide	319		1,920		2,250	
³ tissue water (n = 72)						
chloride (M)	1		1.2		1	
bromide (mM)	6.8		7.6		7.1	
iodide (mM)	1.7		1.8		1.9	

¹ Tissue and soil levels are means from sampling over 12 months.

² sd are high due to 3 months with extremely high iodide values.

³ Calculated indirectly from tissue halide levels and amount of water removed.

month of October and then dropped again in November 2002. After this drop, tissue I⁻ remained low during the rest of the sampling period. The high I⁻ levels in *Salicornia virginica* tissues from September and October 2002 coincided with extensive flowering.

The molar concentrations of Cl⁻, Br⁻, and I⁻ in plant water over the 12 months (Figures 3b, 4b, 5b) were relatively constant; however, a significant interaction was found among month and elevation. The molar concentration of Cl⁻ (Figure 3b) in plant water increased during the summer months and decreased during the wetter winter months, as seen in percent dry weight. The plant water Cl⁻ concentrations from middle elevation plants during the summer months of June, July, August, and September were significantly greater than the concentration during the winter months of January, February, and March. Plant water Cl⁻ concentrations from the middle sampling area were greater than the other two elevations for a large portion of the sampling period; however, this trend was not reflected in the percent dry weight data.

Plant water Br⁻ concentrations (mM) did not follow the seasonal pattern as did chloride concentrations (Figure 4b). There was, however, a decrease in the molar concentration during the wet winter months of December through March.

The concentration of I⁻ (μM) in the plant water over the 12 months (Figure 5b) showed the same pattern as when expressed in ppm (Figure 5a); orders of magnitude increases in concentrations occurring during the months of September and October. During the rest of

the sampling period, molar concentrations remained low and did not follow a seasonal pattern.

Soil Halide Concentrations

Percent Cl⁻ in the high elevation soil was the most variable of the three halides (Table 2). The middle sampling elevation was consistently lower in Cl⁻ content than the other two elevations, and there was a significant difference between the yearly means of soil Cl⁻ at the middle and low elevations (Table 2). Although soil Cl⁻ concentrations were highly variable, the lowest levels were measured January 2003 after the heavy rains of December, and the low elevation that was least affected by rainfall.

The low elevation soil contained higher levels of Br⁻ than the middle elevation, although there was no significant difference between the yearly means of soil Br⁻ at all three elevations (Table 2). There was a trend for the levels to be lower in January 2003 after the wet December, as was seen with Cl⁻, and no significant increase in Br⁻ was seen in soils from the middle and high elevations during the summer. The mean soil I⁻ concentration was consistently higher in the low sampling area compared with the other elevations over the four months sampled; the yearly mean of soil I⁻ from the low elevation was statistically higher than the yearly mean at the middle and high elevations (Table 2). The middle and high sampling elevations fluctuated among months, but for all three elevations, the lowest soil I⁻ concentration occurred in January 2003.

Table 3. Mean molar halide ratios in plant water, soil and seawater.

Halide Pair	Plant Water	Soil	Seawater
Cl:Br	149	153	643
Cl:I	605	1.1×10^5	5.4×10^6
Br:I	4.0	725	8.2×10^3

Plant, Soil and Seawater Halide Relationships

On a dry weight basis, the levels of all three halides were greater in *Salicornia* tissue than in marsh soil (Table 2). The degree of concentration is $I^- > Br^- > Cl^-$. The molar halide concentrations in tissue water shown in Table 2 are also much greater than those found in seawater: 540 mM Cl^- , 0.82 mM Br^- and 100 nM I^- (Brewer 1975). Molar halide ratios (i.e., Cl:Br:I) from all elevations were lower in *S. virginica* tissue than in seawater and soil (mean of all elevations), indicating the selective uptake of halides in the order $I^- > Br^- > Cl^-$ (Table 3). Molar halide ratios in soil from all elevations were also lower as compared to seawater (Table 3), indicating processes favoring retention of I^- over Br^- and Cl^- , and Br^- over Cl^- in marsh soil.

Correlations with Environmental Factors

Of all the analyses conducted, only a single variable showed a significant linear correlation. The correlation between the tissue Cl^- concentration (% DWt and M) at the low and middle sampling areas and the mean water temperature was significant at $\alpha = 0.10$. Other correlations showed no significant relationship between the tissue levels and environmental factors.

DISCUSSION

Halophyte succulence is increased when the plants are exposed to increased salinities up to the limit of their salt tolerance. Any further salinity increase will cause the amount of tissue water to drop (decrease succulence; Naidoo and Rughunanan 1990). Increases in succulence allow the plant to cope with high salinities by diluting ions sequestered in the vacuole with a large proportion of cell water (Jennings 1968). Changes in succulence appear to be determined primarily by Cl^- concentration, followed by that of Na^+ ion concentration (Chapman 1942, Austenfeld 1974).

Succulence of *S. virginica* tended to decrease (% DWt increase) during the dry months of July through October, perhaps in response to the slightly higher soil salinities as reflected by higher, although not significant, soil Cl^- levels during July through October. Succulence increased with increasing monthly rainfall in

response to a return to optimum salt-tolerant growth. Plant tissue from the middle elevation consistently had lower succulence than tissue from the other two elevations, and the average soil Cl^- content was lower than at the other elevations. *Salicornia* may have been growing near its salt tolerance optimum at the low and high locations. Greenhouse-grown *S. bigelovii* showed maximum succulence (7.7 % DWt) and growth at 200 mM NaCl (Ayala and O'Leary 1995). Succulence and growth were reduced at low (5 mM) and high (600 mM) NaCl concentrations. Similarly, greenhouse-grown *S. rubra* A. Nels, an inland species found in salt playas of the Great Basin Desert, showed a maximum succulence at 400 mM NaCl (~1.4% Cl^-), with an approximate 30% drop at 200 mM and 1 M (Khan et al. 2001). In contrast, greenhouse-grown coastal *S. europaea* displayed similar succulence over a large range of NaCl concentrations (0, 85, and 170 mM), decreasing at 340 mM (Moghaieb 2004). For reference, seawater has a Cl^- concentration of 546 mM and a sodium concentration of 463 mM (Brewer 1975).

The halide concentrations in plant water were much greater than halide concentration in seawater, supporting the conclusion that *Salicornia* does not effectively block salt uptake and that it relies on succulence to survive in saline conditions. Plant water halide concentrations are probably less than their concentration in the vacuolar water because this calculation was based on the removal of all cell water (vacuolar and cytoplasmic water). The Cl^- values have been confirmed by analysis of water directly pressed from fresh tissue. Although *Salicornia* does partially exclude some ions from entering the plant (Mahall and Park 1976), those that do enter are stored in high concentrations in the cell vacuole. There is selective uptake of halides in the order $I^- > Br^- > Cl^-$ as indicated from a comparison of the plant water halide molar ratios to those in seawater and soil in Table 3. Soil halide ratios were also lower in the soil compared to seawater. This decrease could be a result of several factors including soil texture and chemistry, vegetation, and proximity to the water table (Mitsch and Gosselink 2000).

Tissue Cl^- levels varied slightly over each month and elevation. Tissue Cl^- levels appear to reflect the seasonal rainfall patterns, with an increase in tissue concentration and plant water concentration during the dry summer months and a decrease during the wet winter months of January, February, and March. Tissue Cl^- levels measured in *Salicornia virginica* from this study were similar to Cl^- levels found in other halophytes. Chapman (1942) found that tissue Cl^- levels in coastal marsh (Norfolk, England) species *S. herbacea* ranged from a high of 24% DWt to a low of 10% from April through October. The profile was very

similar to that of this study, with a maximum near June and slowly declining thereafter, although rainfall patterns were undoubtedly different. Keiffer and Ungar (1997) reported that *S. europaea* tissue Cl^- concentration ranged from 9.2 to 15.6 % DWt (expressed as $\text{mmol } 100 \text{ g}^{-1}$ tissue, converted to % DWt using their reported 82–85% water content), which is generally lower than Cl^- tissue levels found in this study. They found that growth medium salinity (0.5, 1.5, 2.5%) had no effect on biomass, while plant density had a large effect.

Lower soil Cl^- levels measured in January reflected the influx of rain water, which diluted pore water constituents at the middle and high elevations. Correspondingly, tissue Cl^- levels were also low in January through March 2003. Presumably, the higher Cl^- concentrations in the middle and high elevation soil during April through July were a result of evaporation and the lack of rainfall during the winter of 2001–2002 (10.2 cm November through March compared with 31.3 cm for same period during 2002–2003; Orange County Watershed and Coastal Resources Division 2004).

Chloride tissue levels and plant water concentrations began to increase uniformly from May to June, reflecting the increase of Cl^- in the soil and corresponding to a decrease in succulence. In coastal salt marsh soils seemingly equivalent to our low or middle levels, Chapman (1942) measured Cl^- concentrations ranging from 2 to 3%, with minimal correspondence between *Salicornia* tissue and soil Cl^- concentrations. Those values and the weight-based tissue/soil Cl^- ratios ranging from 5 to 12, were very similar to those reported here. Coastal salt marsh soils contain at least two orders of magnitude more Cl^- than soils away from the coastline (Yuita 1983), reflecting the tidal influence.

To our knowledge, there are no previous reports on the Br^- content of coastal marsh plants except Market and Jayasekera (1987), who reported concentrations (as total bromine by atomic absorption spectrophotometry, inductively coupled plasma emission spectroscopy, and neutron activation analysis) ranging from 0.01 to 0.4 % DWt in the red mangrove, *Rhizophora mangle* and sea aster *Aster tripolium*. These values are lower than those reported herein for *S. virginica*. As with Cl^- , the levels of Br^- in tissue were at least 10 times greater than the concentration of Br^- in glycophyte tissue (Market and Jayasekera 1987). Bromide levels in rice (*Oryza sativa* (L.)) leaves grown in coastal regions of Japan ranged from 4.2×10^{-4} to 3.7×10^{-3} % DWt (Yuita et al. 1982a). In our study, Br^- tissue levels and concentrations in plant water did not display a definite seasonal trend like that of Cl^- and varied only slightly during the sampling period. The month that showed the highest variation was April

2002, after which tissue Br^- remained stable. Tissue Br^- levels did not correlate with soil Br^- concentrations but were approximately 10x greater than soil Br^- .

Soil Br^- concentrations in the marsh were moderately stable over the sampling period. Unlike the trend in soil Cl^- levels, Br^- levels from the middle and high elevations did not tend to increase during the dry summer period. This was surprising because pore water ion concentrations at the middle and high elevations should have increased during the dry months because of evaporation, while the salinity at the low elevation should have remained relatively constant because of daily tidal inundation (Mitch and Gosselink 2000). Bromide levels in the marsh soil were two orders of magnitude greater than non-marsh soils. Soil from a coastal plain in Japan, 2 km from the sea, had a Br^- level of 8.7×10^{-4} % (Yuita 1983), and coastal sand dunes had a Br^- level of 5.9×10^{-4} % (Yuita et al. 1982a), values very similar to those reported here.

The mean *Salicornia* tissue I^- levels were well above the only other known published values for halophytes. Iodine tissue levels from the red mangrove, *Rhizophora mangle* and sea aster, *Aster tripolium* ranged from 6 to 8 ppm (Market and Jayasekera 1987). The tissue I^- levels from rice (*Oryza sativa*, a glycophyte) leaves grown in I^- rich soil in Japan ranged from 0.22 to 2.3 ppm (Yuita et al. 1982a); however, values of 13 ppm were recorded in rice leaves from plants grown in elevated I^- soils (Redeker et al. 2004). Tissue I^- levels and plant water concentrations were highly variable, with three of the twelve months (April, September, and October 2002) showing extremely high concentrations. The high I^- concentrations in *S. virginica* tissues from September and October 2002 coincided with extensive flowering and not elevated soil concentrations. *Salicornia virginica* concentrates I^- in its tissues, 10 to 1000x that found in marsh soils.

The low elevation soil contained much more I^- than both the middle and high sampling elevations throughout the study period. The lowest soil I^- concentration was measured at the high elevation in January of 2003, perhaps reflecting dilution by the large amount of rainfall and oxidative conditions favorable to IO_3^- formation. This is consistent with other studies, which found that the reduced soils of marshes decrease the amount of IO_3^- and increase the amount of I^- in the water (Luther and Cole 1988, Smith and Butler 1990). Also, higher I^- soil levels in the low elevation during the spring/summer, as compared to fall/winter, probably reflect an increase in the biological and chemical reduction of IO_3^- to I^- .

Studies performed in I^- rich soils in Japan showed that the concentrations of I^- in those soils were approximately the same as in marsh soils: 1.7 ppm I^- in

coastal plain soil (Yuita 1983), 3.1 ppm I^- in alluvial plain soil and 3.6 ppm I^- in coastal sand dunes (Yuita et al. 1982a,b). These concentrations are approximately 10 times higher than other soils around the world.

The levels of Cl^- and Br^- in the soil at the low elevation were expected to be lower than those at the higher elevations because of evaporative concentration of ions at these less tidally influenced elevations. This, however, was not the case; most of the values at the low elevation were equal to or greater than the higher elevations. Although rainfall may have leached halides from the soils at the elevated sites during the rainy season, this does not explain the lowered values of Cl^- and Br^- compared to the low elevation soil for the rest of the year. In addition to the leaching effects of rainfall, perhaps plant and microbial halide removal is significant at the higher elevations.

Rainfall may affect soil I^- differently than the other two halides. In addition to leaching I^- from the soil, rainfall may inject highly oxygenated water into the soil, which would favor the conversion of I^- to IO_3^- and have no effect on the Cl^- or Br^- present. As intervals between rainfalls increased, I^- would return to the soil from tidal inundation and become more concentrated due to evaporation (primarily high elevation) and reduction of IO_3^- (primarily low elevation). The soil in the low sampling area should maintain a high I^- concentrations because of the anoxic, reducing conditions in this area. The low elevation soil consistently contained more I^- than the other elevations. Tissue from the low sampling area, however, did not contain more I^- than the other areas. This may be a result of uptake being saturated at the soil I^- concentrations encountered or significant removal of I^- from the tissue as volatile CH_3I as is seen in rice (Redeker et al. 2002).

Tissue levels of Cl^- , Br^- , and I^- did not correlate with their respective soil concentrations. The lack of correlation may reflect the high soil concentrations to which the plants were exposed and the continual uptake of water and halides. Tissue Cl^- in the low and middle sampling elevations showed a significant positive correlation with water temperature ($p=0.04$ and 0.09 , respectively); however, increased water temperature did not correlate with Br^- and I^- tissue levels at these elevations. Dalton and Gardener (1978) demonstrated that the transpiration rate (and thus, water-solute uptake) increases with an increase in temperature. *Salicornia* has no active mechanism to rid itself of excess salt. Only the production of new tissue effectively counters continual salt uptake. Eventually, the fleshy stem will become woody, with the plant being primarily woody in the non-growing months of winter. It would have been informative to have measured halides in this woody tissue to see if the levels were equal to or less than green growing tissue.

Sequestration and Volatilization of Halides by Halophytes

Like other succulent halophytes, *S. virginica* concentrates Cl^- in its tissues, presumably in its vacuolar water. The concurrent concentration of Br^- and I^- has not been previously reported. *Salicornia virginica* selectively sequesters these halides in its tissues, favoring I^- over Br^- and Br^- over Cl^- . Further discrimination occurs when these halides are emitted by the plant into the atmosphere as volatile methyl halides, as shown by the molar emission ratio of 20: 1, CH_3Cl to CH_3Br (Rhew et al. 2000) as compared to the molar ratio of approximately 150: 1 plant tissue water (Table 3). Although CH_3I emissions have been detected from halophytes including *Salicornia* (Manley, unpublished), emission rates of CH_3I have yet to be reported. The high I^- tissue values sampled in both September and October correspond with extensive flowering in *Salicornia*, and it is possible that this increase was a result of a change in the physiological status associated with flowering affecting the efficiency of I^- uptake. Increases in methyl iodide production by *Salicornia* during the flowering season may reflect this increase in tissue iodide levels (Manley unpublished). Methyl transferases are the enzymes responsible for the methylation of halides in plants, and a methyltransferase has been isolated from petals of the native California plant, *Clarkia breweri* (A. Gray) Greene, that functions to create the volatile compounds associated with this flower (Ross et al. 1999). *Salicornia virginica* may also produce a methyltransferase that utilizes excess I^- in tissue during the flowering season. The strength of methyl halide production suggests that not all halides present in the plant tissue are "metabolically inert." Either methyltransferase and SAM are present in the vacuole for halide methylation or some halides must be present outside the vacuole and accessible to the appropriate methyltransferase. Methyl halides emission, however, is not a significant method of halide excretion for another salt marsh halophyte, *Batis* (Manley 2002).

Because coastal halophytes are globally significant producers of atmospherically important methyl halides, further physiological and biochemical studies of plant halogen uptake and assimilation are necessary to improve characterization of this process and the role of these plants in halide biogeochemistry.

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