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Highlights

Total ions, Cl^- and Na^+ increased with higher salinity in leaves and rhizomes of both seagrass species.

K^+ and Ca^{+2} generally decreased with higher salinity.

Cl^- and Na^+ were higher in rhizomes than in leaves, possibly reflecting effective ion exclusion mechanisms in leaves.

The ratios K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$ were higher in *H. wrightii* than in *T. testudinum* leaves.

Short-term impacts of salinity pulses on ionic ratios of the seagrasses *Thalassia testudinum* and *Halodule wrightii*

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Abstract

We examined the effects of short-term salinity pulses on ion accumulation in the seagrasses *Thalassia testudinum* and *Halodule wrightii*. Plant fragments were exposed for approximately one week to 10, 23 (ambient salinity), 30, 40, 50 and 70 psu. The concentrations of total ions, Cl^- and Na^+ increased with higher salinity in leaves and rhizomes of both seagrass species. In contrast, the concentrations of K^+ and Ca^{+2} generally decreased with higher salinity, although the decrease was relatively small and only observed at extreme salinities. Our results indicate the concentrations of Cl^- and Na^+ were higher in rhizomes than in leaves, possibly reflecting effective ion exclusion mechanisms in leaves. Under ambient (control) salinity the ratios K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$ were 38% and 46% higher in *H. wrightii* than in *T. testudinum* leaves, which support the notion that *H. wrightii* is more tolerant of salinity increases than *T. testudinum*. In concert, our results show novel observations of ion osmolyte concentrations in these seagrass species that point to adaptive responses to salinity pulses. Despite these adaptive responses, pulses of extremely high salinity (>50 psu) lasting approximately one week are detrimental to these seagrass species.

Key words

Seagrass; Salinity stress; Ions; *Thalassia testudinum*; *Halodule wrightii*

1. Introduction

Seagrasses are a unique group of angiosperms that grow completely submerged in seawater, where NaCl is the dominant salt present. Indeed, the tolerance of seagrass species to high salinity has often been proposed as the reason for their occurrence in seawater (Tyerman, 1989). Seagrasses exposed to high salinity (high NaCl) regulate water and osmolyte balance through osmotic adjustment.

Osmotic adjustment maintains cell water contents by increasing the osmotic force that can be exerted by cells on their surroundings, which increases water uptake. This process entails an increase in solute content and concomitant decrease in osmotic potential ($\Psi\pi$) (Tyerman, 1989; Touchette, 2007). The initial hyperosmotic response (minutes to hours to days) to increased salinity typically involves raising ion levels within cells by accumulating ions from the external environment until osmotic equilibrium is achieved (Flowers et al., 1977; Tyerman, 1982; Touchette, 2007). Seagrasses can accumulate salt in concentrations equaling or exceeding those in seawater without any harmful impacts on the plant (Flowers et al., 1977). Specifically, seagrasses accumulate sodium (Na^+), potassium (K^+) and chloride (Cl^-) (Touchette, 2007) and these concentrations may vary among seagrass species and organs of the same plant (Birch, 1975; Ye and Zhao, 2003).

Ions are very efficient osmolytes, the cellular concentrations of which can be adjusted easily and quickly with low metabolic energy costs, especially compared to the costs involved in biosynthesis or degradation of organic osmolytes (Raven, 1985). Aquatic plants can take up ions via roots, shoots and leaves (Babourina and Rengel, 2010), and maintenance of adequate levels of K^+ and Ca^{+2} is essential for plant survival. Seagrass cells store Cl^- and Na^+ in vacuoles to avoid elevated cytoplasmic concentration and resulting physiological damage (Carpaneto et al., 1997; Ye and Zhao, 2003; Touchette, 2007). When Cl^- and Na^+ are sequestered in the cell vacuole, K^+ and other organic osmolytes accumulate in the cytoplasm and balance the cell osmotic pressure (Davison and Reed, 1985; Kirst, 1989; Touchette, 2007). Thus, vacuolar ion accumulation contributes to both osmotic adjustment and the maintenance of low cytoplasmic ion concentrations that prevent physiological damage (Hajibagheri and Flowers, 1989). The capacity of plants to counteract salinity stress strongly depends on the status of their K^+ and Ca^{+2} nutrition (Maathuis and Amtmann, 1999). Seagrass species that maintain higher K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$ ratios in the cell under ambient conditions are more tolerant of high salinity conditions (Maathuis and Amtmann, 1999; Muramatsu et al., 2002).

Halodule wrightii (shoalgrass) is a narrow-leaved seagrass considered somewhat more euryhaline than *Thalassia testudinum* (turtlegrass). *H. wrightii* can tolerate salinities as low as 5 psu and as high as 65 psu (Dawes, 1987; Vermaat et al. 1993; Dunton, 1996; Koch et al., 2007). There are several

studies of the influence of salinity on these seagrasses, ranging from impacts on growth and survivorship (McMillan and Moseley, 1967; McMahon, 1968; McMillan, 1974; Lirman and Cropper, 2003; Koch et al., 2007) to physiological parameters, carbohydrates, and osmolality (Berns, 2003; Koch et al., 2007), and seedling performance (Kahn and Durako, 2006). However, few studies have investigated the response of ion concentrations and the function of inorganic solutes in osmotic adjustment in seagrasses (Tyerman, 1982; Tyerman et al., 1984; Ye and Zhao, 2003; Touchette, 2007; Marín-Guirao et al., 2013), and to our knowledge, there are no studies to date. The purpose of the present study was to examine the short-term impacts of salinity pulses on inorganic ion concentrations in leaves and rhizomes of the seagrasses *T. testudinum* and *H. wrightii*.

2. Materials and Methods

2.1 Plant sampling and experimental design

Rhizome fragments of both species with connected shoots and roots were collected from a shallow bed (1-1.5 m depth) at Big Lagoon (Florida) in October 2009. Plants were transported to the Dauphin Island Sea Lab (Alabama, USA) submerged in coolers and transplanted into 19 l (50 cm depth) experimental aquaria within 4 hours of collection. There was no acclimation period since we intended to replicate the impacts of a sudden, abrupt salinity shift (see below). Sediment was obtained from the seagrass collection site and placed into the aquaria. Each aquarium contained four intact rhizome fragments of each seagrass species, with each rhizome fragment having 6-15 connected shoots.

To maintain ambient seawater temperature, the aquaria were submerged in an outdoor flow-through system with pumped seawater that flowed around the aquaria and covered about $\frac{3}{4}$ of their height. In general, the ambient conditions of temperature, salinity and nutrients between the water and seagrass collection sites were similar (Stutes et al., 2007, Cebrian et al., 2013). The water in the aquaria was oxygenated with air pumps and seagrasses were exposed to the following salinity treatments for six (*T. testudinum*) and seven (*H. wrightii*) days: 10, 23 (ambient salinity and control treatment), 30, 40, 50 and 70 psu with three replicated aquaria per treatment. Salinity was increased by adding salt to ambient seawater and decreased by adding distilled water. The changes were sudden, i.e. plants

transported from the field were not acclimated through progressive salinity changes, but subjected to these changes abruptly. Treatments were randomly assigned to aquaria. Salinity levels were maintained within ± 1.5 psu throughout the experiment.

2.2 Plant response measurements

The seagrass *T. testudinum* was sampled 6 days, , and *H. wrightii* 7 days, after starting the experiment, since it was not possible to process samples for the two species on the same day. Leaf and rhizome cation (Na^+ , K^+ , Ca^{+2}) and anion (Cl^-) concentrations were determined using an ion chromatograph Metrohm 850 ProFlC AnCat- MCS with chemical suppression and conductimetric detection. Analyses were performed on two samples per species and aquaria. Each leaf sample consisted of the basal section (15 cm long) of the second youngest leaf for two *T. testudinum* shoots and four *H. wrightii* (two sections from two leaves from different shoots for *T. testudinum*, and four for *H. wrightii*). Rhizome samples consisted on several fragments of rhizome pooled together. The leaf samples were cleaned of epiphytes, samples were rinsed profusely with freshwater to remove attached salts, and then dried at 80C for 24 hours (Birch, 1975). Subsequently, the samples were digested in 15 ml of a 3.5 mM HNO_3 solution made with ultrapure water, stirred for 30 minutes and centrifuged at 5000 rpm for 5 minutes. The supernatant was first filtered through 0.45 μm and then through a Sep-Pak C-18 column before analysis in the ion chromatograph (Marín-Guirao et al., 2013). Samples of *H. wrightii* rhizome for 30psu and 40psu were lost and are not included in the analysis.

2.3 Statistical analysis

Rhizome ion concentration (for *T. testudinum*) was analyzed using one-way ANOVA with salinity as the single factor. Ion concentrations and ratios in leaves were analyzed with a two-factor ANOVA with salinity and seagrass species as the factors. Post-hoc comparisons were done with the Tukey test. For the two-way ANOVA's, Tukey tests comparing salinity treatments were done after pooling the two species for each salinity treatment if the main salinity effect was significant and the interaction term between salinity and seagrass species was not. If the interaction term was significant, one-way ANOVA's followed by Tukey tests were done among salinity treatments for each seagrass species separately (Sokal and Rohlf, 1998; Quinn and Keough, 2002). Homogeneity of

variance was evaluated with Cochran's test (Underwood, 1997). All samples from the same aquarium were averaged into one single true replicate for analysis. Differences were considered significant at $p \leq 0.05$.

3. Results

Cl^- concentration in leaves increased with increased salinity to a similar extent in both seagrasses by the end of the experiment with no significant interaction between salinity and seagrass species (Figure 1, Table 1). The increase was gradual, with lower salinities (10 and 23 psu) having lower concentrations than mid salinities (30 and 40 psu), which in turn had lower concentrations than high salinities (50 and 70 psu). Na^+ concentrations in leaves at the end of the experiment also increased with increased salinity, and the extent of the increase was similar for the two seagrass species (no significant interaction between salinity and seagrass species; Figure 1, Table 1). Significant differences were found between 10 and 40 psu, and between low/mid salinities (10-40 psu) and high salinities (50-70 psu).

K^+ concentration in leaves at the end of the experiment was lower in the highest salinity treatment (70 psu) than in the rest of treatments for both species (no significant interaction between salinity and seagrass species; Figure 1, Table 1). No differences were found among salinities < 70 psu.

Ca^{+2} concentration in leaves at the end of the experiment decreased with increased salinity for both species with no significant interaction between salinity and seagrass species (Figure 1, Table 1). The decrease was gentle, and significant differences were only found between the lowest (10 psu) and the highest salinity treatments (50 and 70 psu). Total ion concentration in leaves at the end of the experiment increased with increased salinity to a similar extent in both species with no significant interaction between salinity and seagrass species; (Figure 2, Table 1). Significant differences were found between 40 and 10-23 psu, 50 and 10-30 psu, and 70 and 10-40 psu.

The ratio K^+/Na^+ in leaves at the end of the experiment decreased with increased salinity, and that decrease was steeper for *H. wrightii* than for *T. testudinum* (significant interaction between salinity and seagrass species; Figure 1, Table 1). Namely, a rather gradual decrease was found for *H.*

wrightii from the lowest to the highest salinity treatment (i.e. 23 was lower than 10 psu, 30 lower than 10 psu, 40 lower than 10 and 23 psu, 50 lower than 10-30 psu, and 70 lower than 10-40 psu), but for *T. testudinum* the ratio was lower in the highest salinity treatment than in the rest, lower in 50 than in 10-40 psu, and no significant differences were found among the treatments 10-40 psu. Similar results were observed for the $\text{Ca}^{+2}/\text{Na}^{+}$ ratio in leaves at the end of the experiment; the ratio decreased with increased salinity but the decrease was steeper for *H. wrightii* than for *T. testudinum* with a significant interaction between salinity and seagrass species; (Figure 1, Table 1). Under ambient conditions (i.e. control salinity treatment of 23 psu), *H. wrightii* displayed higher $\text{K}^{+}/\text{Na}^{+}$ and $\text{Ca}^{+2}/\text{Na}^{+}$ ratios in its leaves than did *T. testudinum* (Tukey tests between the two species at 23 psu for each ratio, $P \leq 0.05$).

Similar results were found for Cl^{-} and Na^{+} concentrations in *T. testudinum* rhizomes as salinity increased. At the end of the experiment these concentrations increased with increased salinity in a rather gradual manner (Figure 3, Table 1), with the concentration lowest at 10 psu and becoming progressively higher at higher salinities. K^{+} concentrations in *T. testudinum* rhizomes at the end of the experiment showed higher values at mid (30-40 psu) than at low and high salinities (10, 50 and 70 psu; Figure 3, Table 1). A slight decrease with increased salinity was found for Ca^{+2} in *T. testudinum* rhizomes at the end of the experiment, with 50 and 70 psu being significantly lower than 10 psu (Figure 3, Table 1). Total ion concentration in *T. testudinum* rhizomes at the end of the experiment increased with increased salinity, with 40 psu displaying significantly higher concentrations than 10-23 psu, 50 higher values than 10-30, and 70 higher values than 10-40 (Figure 2, Table 1). Both $\text{K}^{+}/\text{Na}^{+}$ and $\text{Ca}^{+2}/\text{Na}^{+}$ ratios in *T. testudinum* rhizomes at the end of the experiment decreased with increased salinity. The decrease was gradual with progressively lower values as salinity increased (Figure 3, Table 1). Despite the limited sample size for *H. wrightii* rhizomes, our results show that, for any given salinity level, Cl^{-} and Na^{+} concentrations in rhizomes were higher than in leaves for both seagrass species.

4. Discussion

The Na^+ and K^+ concentrations measured in our study were similar to the mean values reported by Touchette (2007) for various seagrass species (Na^+ : $28.9 \pm 10.8 \text{ mg g}^{-1}$; K^+ : $17.6 \pm 2.7 \text{ mg g}^{-1}$). The increase in total ion concentration for seagrass leaves and rhizomes was due to the increase in the ions with major osmotic activity (i.e. Na^+ and Cl^-). As shown by other studies with seagrass and marine algae, these ionic accumulations could be achieved by processes such as passive diffusion and active transport, (Blumwald et al., 2000), and/or seagrass-specific channel-based transportation mechanisms (Carpaneto et al., 2004; Babourina and Rengel, 2010). Ion accumulation could cause osmotic adjustment, decreasing the osmotic potential ($\Psi\pi$) (Tyerman, 1989; Touchette, 2007). The accumulation of Cl^- is balanced by the accumulation of Na^+ and K^+ . Large accumulation of Na^+ and Cl^- , however, may be harmful for seagrasses (Greenway and Munns, 1980), and it seems seagrasses store these ions in vacuoles to minimize damage (Binzel et al., 1988; Niu et al., 1995; Storey and Walker, 1998).

A decrease in K^+ levels at high salinities, while Na^+ and Cl^- concentration increased, was also observed in red algae and in the brown algae *Colpomenia* sp. (Kirst and Bisson, 1979). Due to the importance of K^+ and Ca^{+2} in cell integrity, species that maintain higher cell K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$ ratios under ambient conditions are more tolerant of high salinity conditions (Maathuis and Amtmann, 1999; Muramatsu et al., 2002). The higher ratios (K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$) found at 23 psu (ambient salinity in the study site) for *H. wrightii*, than for *T. testudinum* may indicate a better physiological ability of *H. wrightii* to cope with high salinity in comparison with *T. testudinum*. These results provide support to the notion that *H. wrightii* is more tolerant to salinity increases than *T. testudinum* (Koch et al. 2007, Touchette 2007).

Overall ion concentration in the leaves and rhizomes of both seagrass species decreased from Cl^- to Na^+ to K^+ to Ca^{+2} . This differs from past results reported for *Zostera marina*, where concentrations decreased from Na^+ to K^+ to Cl^- to Ca^{+2} in both leaves and rhizomes (Ye and Zhao, 2003). Perhaps ion accumulation processes in *Z. marina* are more similar to marine macroalgae, which also accumulate high Na^+ and K^+ concentrations (Touchette, 2007). In addition, Na^+ , Cl^- and K^+ concentrations were higher in leaves than rhizomes in *Z. marina* (Ye and Zhao, 2003), whereas Cl^- , Na^+ , K^+ and Ca^{+2} concentrations were higher (more than twice) in rhizomes than leaves in *T. testudinum* and *H. wrightii*. The latter may indicate more efficient ion exclusion mechanisms in

leaves for the protection of enzymatic photosynthetic reactions. Despite the restricted sample size for ion concentration in *H. wrightii* rhizomes, the pattern appears to be qualitatively similar to *T. testudinum* rhizomes.

In our study, 50 psu seems to be the salinity that leads to reduced growth and decreased photosynthetic efficiencies (data not shown), presumably due to loss of osmotic balance following accumulation of Cl^- and Na^+ and depletion of K^+ and Ca^{+2} . At this salinity, Na^+ and Cl^- concentrations were much higher than ambient values. It appears this hypersaline condition exceeded the ionic exclusion or accumulation capacity of these species for attaining ion homeostasis (Greenway and Munns, 1980; Gorham et al., 1985). Ion accumulation for maintaining osmotic adjustment is an efficient mechanism to cope with short-term osmotic stress (minutes to hours to a few days) (Flowers et al., 1977; Tyerman, 1982; Touchette, 2007), but it may not be sustainable for several days (Touchette, 2007; Marín-Guirao et al., 2013).

In conclusion, our results show novel observations of ion osmolyte concentrations in cornerstone Gulf of Mexico seagrass species under varying salinities, which point to adaptive processes to salinity pulses. These observations support the notion that *H. wrightii* is more tolerant of salinity increases than *T. testudinum*. Both species adapt well (without major detrimental effects) to salinity values not far from ambient values. At moderate salinity increases, *H. wrightii* does not endure as much damage in morphological/functional traits as does *T. testudinum* (data not shown), as suggested by the higher K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$ ratios in the former species. However, short-term (lasting up to 6-7 days) pulses of extremely high salinity are detrimental to both seagrass species, as reflected in substantial damage on morphological/functional traits (data not shown), decreases in cellular K^+ and Ca^{+2} concentrations, increases in Na^+ and Cl^- concentrations, and decreases in K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$ ratios.

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References

- Babourina, O., Rengel, Z., 2010. Ion transport in aquatic plants. In: Mancuso, S., Shabala, S. (Eds.), *Waterlogging Signalling and Tolerance in Plants*. Springer, Verlag Berlin Heidelberg, pp. 221-238.
- Berns, D.M., 2003. Physiological responses of *Thalassia testudinum* and *Ruppia maritima* to experimental salinity levels. Master of Science Thesis, University of South Florida.
- Binzel, M.L., Hess, D.F., Bressan, R.A., Hasewaga, P.M., 1988. Intracellular Compartmentation of Ions in Salt Adapted Tobacco Cells. *Plant Physiol.* 86, 607-614.
- Birch, W.R., 1975. Some chemical and calorific properties of tropical marine angiosperms compared with those of other plants. *Journal of Applied Ecology*, 12 (1), 201-212.
- Blumwald, E., Aharaon, G.S., Apse, M.P., 2000. Sodium transport in plant cells. *Biochem. Biophys. Acta.* 1465, 140–151.
- Carpaneto, A., Cantù, A.M., Busch H., Gambale, F., 1997. Ion channels in the vacuoles of the seagrass *Posidonia oceanica*. *FEBS Letters* 412, 236–240.
- Carpaneto, A., Naso, A., Paganetto, A., Cornara, L., Pesce, E.-R., Gambale, F., 2004. Properties of ion channels in the protoplasts of the Mediterranean seagrass *Posidonia oceanica*. *Plant Cell Environ.* 27, 279–292.
- Cebrian, J., Stutes, P.J, Christiaen, B., 2013. Effects of grazing and fertilization on epiphyte growth dynamics under moderately eutrophic conditions: implications for grazing rate estimates. *Marine Ecology Progress Series* 474: 121-133.
- Davison, I.R., Reed, R.H., 1985. The physiological significance of mannitol accumulation in brown algae: the role of mannitol as a compatible cytoplasmic solute. *Phycologia* 24, 449-457.
- Dawes, C.J., 1987. The dynamic seagrasses of the Gulf of Mexico and Florida coasts. In: Durako MJ, Phillips RC, Lewis RR III (Eds.) *Proceedings of the symposium on subtropicaltropical seagrasses of the southeastern United States*. Florida Marine Research Publications. Number 42. Florida Department of Natural Resources Bureau of Marine, Research, St. Petersburg, FL, pp. 25–38.

- 1 Dunton, K.H., 1996. Photosynthetic production and biomass of the subtropical seagrass *Halodule*
2 *wrightii* along an estuarine gradient. *Estuaries*. 19, 2B: 436-447.
- 3 Flowers, T.J., Troke, P.F., Yeo, A.R., 1977. The mechanism of salt tolerance in halophytes. *Annu.*
4 *Rev. Plant Physiol.* 28: 89-121.
- 5 Greenway, H., Munns, R., 1980. Mechanisms of salt tolerance in nonhalophytes. *Annual Review of*
6 *Plant Physiology* 31, 149-190.
- 7 Hajibagheri, M.A., Flowers, T.J., 1989. A. Läuchli, U. Lüttge (Eds.), *Salinity: Environment - Plants*
8 *- Molecules*, pp. 423-449.
- 9 Kahn, A.E., Durako, M.J., 2006. *Thalassia testudinum* seedling responses to changes in salinity and
10 nitrogen levels. *J. Exp. Mar. Biol. Ecol.* 335, 1-12.
- 11 Kirst, G.O., 1989. Salinity tolerance of eukaryotic marine algae. *Annu. Rev. Plant Physiol. Mol.*
12 *Biol.* 40. 21-53.
- 13 Kirst, G.O., Bisson, M.A., 1979. Regulation of turgor pressure in marine algae: ions and low-
14 molecular-weight organic compounds. *Aust. J. Plant Physiol.* 6, 539-56.
- 15 Koch, M.S., Schopmeyer, S.A., Kyhn-Hansen, C., Madden, C.J., Peters, J.S, 2007. Tropical seagrass
16 species tolerance to hypersalinity stress. *Aquatic Botany* 86, 14-24.
- 17 Lirman, D., Cropper, W.P., 2003. The influence of salinity on seagrass growth, survivorship, and
18 distribution within Biscayne Bay, Florida: field, experimental, and modeling studies. *Estuaries*
19 26(1), 131-141.
- 20 Maathuis, F.J.M., Amtmann, A., 1999. K^+ nutrition and Na^+ toxicity: the basis for cellular K^+/Na^+
21 ratios. *Ann. Bot.* 84, 123-133.
- 22 McMahon, C.A., 1968. Biomass and salinity tolerance of shoalgrass and manatee grass in Lower
23 Laguna Madre, Texas. *J. Wildl. Manage.* 32, 501-506.
- 24 McMillan, C., Moseley, F.M., 1967. Salinity tolerances of five marine spermatophytes of Redfish
25 Bay, Texas. *Ecology* 48, 503-506.

- 1 McMillan, C., 1974. Salt tolerance of mangroves and submerged aquatic plants. In Ecology of
2 Halophytes. Reimold, R.J.; Queen, W.H. (Eds.), Academic Press: New York, pp. 379-390.
- 3 Marín-Guirao, L., Sandoval-Gil, J.M., Bernardeau-Esteller, J., Ruíz, J.M., Sánchez-Lizaso, J.L.,
4 2013. Responses of the Mediterranean Seagrass *Posidonia oceanica* to hypersaline stress duration
5 and recovery. Marine Environmental Research 84, 60–75.
- 6 Muramatsu, Y., Harada, A., Ohwaki, Y., Kasahara, Y., Takagi, S., Fukuhara, T., 2002. Salt-
7 Tolertant ATPase Activity in the Plasma Membrane of the Marine Angiosperm *Zostera marina* L.
8 Plant Cell Physiol. 43 (10), 1137-1145.
- 9 Niu, X., Bressan, R.A., Hasegawa, P.M., Pardo, J.M., 1995. Ion homeotasis in NaCl stress
10 environments. Plant Physiol. 109, 735–742.
- 11 Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge
12 University Press, Cambridge, UK, 537 pp.
- 13 Raven, J.A., 1985. Energetics and transport in aquatic plants. Alin R. Liss, Inc., New York, NY.
- 14 Sokal, R.R., Rohlf, F.J., 1998. Biometry. W. H. Freeman and Company, New York, USA, pp. 887.
- 15 Storey, R., Walker, R.R., 1998. Citrus and salinity. Science Horticulturae 78 (1-4), 39-81.
- 16 Stutes, J. P., Cebrian, J., Stutes, A.L., Hunter, A., Corcoran, A.A., 2007. Benthic metabolism across
17 a gradient of anthropogenic impact in three shallow coastal lagoons in NW Florida. Mar. Ecol.
18 Progr. Ser. 348: 55-70.
- 19 Touchette, B.W., 2007. Seagrass-salinity interactions: physiological mechanisms used by submersed
20 marine angiosperms for a life at sea. Journal of Experimental Marine Biology and Ecology 350, 194-
21 215.
- 22 Tyerman, S.D., 1982. Stationary volumetric elastic modulus and osmotic pressure of the leaf cells of
23 *Halophila ovalis*, *Zostera capricorni*, and *Posidonia australis*. Plant Physiol. 69, 957-965.
- 24 Tyerman, S.D., 1989. Solute and water relations of seagrasses. In: Larkum A.W.D., Mc Combo
25 A.J., Sherpherd S.A. (Eds.) Biology of Seagrasses: a Treatise on the Biology of Seagrasses with
26 Special Reference to the Australian Region. Elsevier, Amsterdam, pp. 723-759.

Tyerman, S.D., Hatcher, A.I., West, R.J., Larkum, A.W.D., 1984. *Posidonia australis* growing in altered salinities: leaf growth, regulation of turgor and the development of osmotic gradients. Aust J Plant Physiol. 11, 35-47.

Underwood, A. J., 1997. Experiments in Ecology. Their logical design and interpretation using analysis of variance. Cambridge University Press. Cambridge, Reino Unido, pp.504.

Vermaat, J. E., Beijer, J. A. J., Gijlstra, R., Hootsmans, M. J. M, Philippart, C. J. M. , van den Brink, N. W., van Vierssen, W., 1993. Leaf dynamics and standing stocks of intertidal *Zostera noltii* Hornem. and *Cymodocea nodosa* (Ucria) Ascherson on the Banc d'Arguin (Mauritania). Hydrobiologia 258: 59-72

Ye, C.J., Zhao, K.F., 2003. Osmotically active compounds and their localization in the marine halophyte egrass. Biol. Plant. 46, 137-140.

Fig.1. The effects of salinity on leaf ion concentration (mg g^{-1}) and ratios K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$. Solid circles correspond to *T. testudinum* and open circles to *H. wrightii*. Values are to means values and confidence intervals are S.E. Letters denote the results of post-hoc Tukey comparisons (one-way ANOVA or two-way ANOVA with non-significant interaction term). Insets of ordered salinity treatments denote the results of post-hoc Tukey comparisons for each species (two-way ANOVA with significant interaction term). See text for details.

Fig.2. The effects of salinity on total ion concentration (mg g^{-1}) in (A) leaves and (B) rhizome. Solid circles correspond to *T. testudinum* and open circles to *H. wrightii*. Values are to means values and confidence intervals are S.E. Letters denote the results of post-hoc Tukey comparisons (one-way ANOVA or two-way ANOVA with non-significant interaction term). Due to equipment failure only

one replicate for some of the salinity treatments existed for *H. wrightii*. Such values are plotted for illustrative purposes. See text for details.

Fig.3. The effects of salinity on rhizome ion concentration (mg g^{-1}) and ratios K^+/Na^+ and $\text{Ca}^{+2}/\text{Na}^+$. Solid circles correspond to *T. testudinum* and open circles to *H. wrightii*. Values are to means values and confidence intervals are S.E. Letters denote the results of post-hoc Tukey comparisons. Due to equipment failure only one replicate for some of the salinity treatments existed for *H. wrightii*. Such values are plotted for illustrative purposes. See text for details.

Table1. ANOVA summary table comparing variations in ions concentrations in leaves for *T. testudinum* and *H. wrightii* and rhizome concentrations for *T. testudinum*.

Variable	Effect	df	MS	F	p
Leaves					
Cl ⁻	Sal	5	6.8	41.0	≤0.05
	Species	1	0.2	1.5	0.25
	SalxSpecies	5	0.1	0.9	0.50
	Residual	12	0.2		
	Total	23			
Na ⁺	Sal	5	5.0	82.7	≤0.05
	Species	1	3.2	52.1	≤0.05
	SalxSpecies	5	0.1	1.0	0.47
	Residual	12	0.1		
	Total	23			
K ⁺	Sal	5	22.4	15.7	≤0.05
	Species	1	21.5	15.1	≤0.05
	SalxSpecies	5	1.6	1.1	0.40
	Residual	12	1.4		
	Total	23			
Ca ⁺²	Sal	5	0.8	7.5	≤0.05
	Species	1	1.4	12.3	≤0.05
	SalxSpecies	5	0.3	2.7	0.07
	Residual	12	0.1		
	Total	23			
Total	Sal	5	2937.1	25.2	≤0.05
	Species	1	685.1	5.9	≤0.05
	SalxSpecies	5	71.3	0.6	0.69
	Residual	12	116.7		
	Total	23			
K ⁺ /Na ⁺	Sal	5	0.2	198.3	≤0.05
	Species	1	0.3	329.2	≤0.05
	SalxSpecies	5	0.0	28.3	≤0.05
	Residual	12	0.0		
	Total	23			
Ca ⁺² /Na ⁺	Sal	5	0.0	474.6	≤0.05
	Species	1	0.0	594.4	≤0.05
	SalxSpecies	5	0.0	109.8	≤0.05
	Residual	12	0.0		
	Total	23			
Rhizomes					
Cl ⁻	Sal	5	3754.6	127.5	≤0.05
	Residual	6	29.4		
	Total	11			
Na ⁺	Sal	5	1722.6	132.5	≤0.05
	Residual	6	13.0		
	Total	11			
K ⁺	Sal	5	43.0	14.6	≤0.05
	Residual	6	2.9		
	Total	11			
Ca ⁺²	Sal	5	0.7	5.1	≤0.05
	Residual	6	0.1		
	Total	11			
Total	Sal	5	10164.1	87.6	≤0.05
	Residual	6	116.0		
	Total	11			
K ⁺ /Na ⁺	Sal	5	0.1	56.6	≤0.05
	Residual	6	0.0		
	Total	11			
Ca ⁺² /Na ⁺	Sal	5	0.0	82.8	≤0.05
	Residual	6	0.0		
	Total	11			

Table1. ANOVA summary table comparing variations in ions concentrations in leaves for *T. testudinum* and *H. wrightii* and rhizome concentrations for *T. testudinum*.