Journal of Experimental Marine Biology and Ecology 386 (2010) 34–38

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Sensitivity of the seagrass *Cymodocea nodosa* to hypersaline conditions: A microcosm approach

Jordi F. Pagès [a,*], Marta Pérez [b], Javier Romero [b]

[a] Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Accés a la cala Sant Francesc, 14, 17300 Blanes, Girona, Spain
[b] Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona. Av. Diagonal, 645, 08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 10 August 2009
Received in revised form 18 February 2010
Accepted 19 February 2010

Keywords:
Cymodocea nodosa
Desalination impact
Hypersalinity stress
Mediterranean Sea
Pam fluorometry

ABSTRACT

Marine water desalination is an emergent strategy to satisfy water demand in coastal regions. The brine originated as a by-product (60–90 psu) can have a significant impact on important marine ecosystems, yet the tolerance thresholds of most key species remain unknown. Therefore, with the aim of filling this gap and providing useful information to mitigate such impacts, we have experimentally assessed the response to hypersaline conditions of the seagrass *Cymodocea nodosa*. We exposed plants in microcosms to 4 treatments: control (37.2 psu), 44 psu, 54 psu and 62 psu, with three replicate aquaria per treatment for 17 days, and we measured plant response and status. We found that the seagrass shoots endured 44 psu without any apparent damage, but vitality decreased in 54 and 62 psu treatments. Plants at both 54 and 62 psu displayed reduced leaf growth and higher incidence of leaf necrosis, relative to control conditions. In addition, in plants at 62 psu, photosynthetic performances (measured as leaf quantum efficiency) dropped shortly after salt addition, and by the end of the experiment they also suffered substantial mortality. These findings should be viewed with caution, due to the relatively short time-span of the experiment, as in the long-term plants may be less tolerant than reported here. However, our results highlight the need for careful management of brine disposal to prevent deterioration of seagrass habitats; salinity should never exceed 44 psu inside the meadows, to prevent deterioration of *C. nodosa* habitats.

1. Introduction

Desalinated seawater has become an important source of drinking water in coastal regions where water demand has increased to exceed readily available freshwater resources. This is typically the case of the Mediterranean region, among others (Tsiourtis, 2001). The development of technologies, such as reverse osmosis, with acceptable costs (in terms of energy and space) is seen as a promising solution to water shortages (Torquemada and Lizaso, 2005). However, desalination plants can have significant environmental impacts, mainly due to the large volume of brine (roughly equivalent to the volume of drinking water produced) discharged into the sea, which can reach salinities of between 60 and 90 psu (Lizaso et al., 2008). Less commonly, the chemicals or other materials continuously or sporadically released by these plants (anti-scalant, biocides, surface active agents, solid residues from back flushing of filters, etc.) also cause an environmental impact. The importance of all these impacts depends on the vulnerability of the species and ecosystems receiving the discharge, the general hydrodynamic field, the depth of the discharge and the brine flux, among other factors (Gacia et al., 2007). The effects of hypersaline water have been assessed for some species or communities (e.g. Castriota et al., 2001; Koch et al., 2007a). However, and despite this initial effort, much remains to be learned if we are to properly protect coastal ecosystems against the foreseen increase in desalination activity.

Seagrasses are one of the main targets of such future investigations, for three reasons: (i) their distribution, which overlaps potential discharge points; (ii) their high sensitivity to disturbances; and (iii) their well-known biological and ecological relevance (Green and Short, 2003; den Hartog and Kuo, 2006). Despite recent advances in the field, knowledge of the effects on seagrasses of exposure to high salinities remains restricted to a few species. Seagrasses exposed to changes in salinity can suffer osmotic stress, with the consequent changes at the biochemical and physiological levels (Touchette, 2007). These include alterations of their photosynthetic rates (Biebl and McRoy, 1971; Kerr and Strother, 1985; Dawes et al., 1987) and metabolism (van Katwijk et al., 1999), altered growth rates (McMillan and Moseley, 1967; Walker, 1985; Walker and McComb, 1990), and increased mortality (Vermaat et al., 2000; Torquemada and Lizaso, 2005). Specifically, recent studies have shown very low tolerance thresholds to salinity increases in the late-successional Mediterranean species *Posidonia oceanica* (Torquemada and Lizaso, 2005; Gacia et al., 2007), although little is known concerning other Mediterranean species.

Cymodocea nodosa (Ucria) Ascherson is widely distributed throughout the Mediterranean Sea, NW Africa and South Atlantic

* Corresponding author. Tel.: +34 972 336101; fax: +34 972 337806.
E-mail address: jpages@ceab.csic.es (J.F. Pagès).

0022-0981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2010.02.017

Europe (den Hartog and Kuo, 2006). This species may be considered euryhaline, as it forms healthy stands under a wide range of salinities (Pérez and Romero, 1994; Pérez-Ruzafa et al., 2005), and this suggests it has a higher tolerance to elevated salinities than other seagrass species (e.g. *P. oceanica*). However, the few results available on *C. nodosa* are somewhat controversial. Some preliminary experimental evidence indicates that shoots may suffer deterioration or mortality under hypersaline conditions (Torquemada and Lizaso, 2006), while no significant changes were detected in a field study carried out by Talavera and Ruiz (2001) in a meadow close to the brine discharge from a desalination plant.

With the aim of expanding knowledge of the effects of hypersaline conditions on key organisms, in this study we tested the tolerance of *C. nodosa* to potential brine discharges. We assessed changes in some indicators of plant performance (leaf quantum efficiency, shoot growth, leaf necrosis, shoot mortality and plant biomass) in microcosms where salt concentrations had been manipulated.

2. Materials and methods

2.1. Plant sampling and experimental design

Intact *C. nodosa* shoots (including rhizomes and roots) were carefully harvested in May 2008 from a shallow meadow (approximately 0.3 m deep) in the Badia dels Alfacs; the southern bay of the Ebro Delta (NE Spain). To reduce variability, only shoots less than one year old were collected (i.e. shoots with less than 12 leaf scars on the vertical rhizome; Pérez and Romero, 1994). Thereafter, plants were stored in a large cool container (27 L) with ambient seawater. Sediment was collected from the same site (~40 L) and sieved (~1 mm pore) to exclude macroinvertebrates. Finally, both sediment and plant containers were transported to the Experimental Chambers Service, of the Universitat de Barcelona, where the experiment was conducted.

Within 8 h of collection, the shoots were planted in 12 transparent, cylindrical aquaria (40 cm high × 20 cm in diameter), placed in a controlled environment chamber (2.1 m^2) at light saturation (i.e. ~350 µmol photons m^{-2} s^{-1}; Pérez and Romero, 1992) on a 12 h:12 h light:dark cycle and at constant temperature (close to that in the field at that time of the year, i.e. 19 °C). Independent air pumps were installed to obtain proper aeration at each aquarium. Light and temperature were measured in each aquarium, and revealed some heterogeneity (i.e. ±50 µmol photons m^{-2} s^{-1} and ±1 °C). In each aquarium, 15 shoots (with their corresponding rhizomes and roots) were inserted in 10 cm of sediment and the aquarium was filled with filtered seawater (salinity 37.2 psu) from the ICM–CSIC water services (ZAE). Additional shoots ($n = 15$) were sorted and dried to obtain initial biomass (see below).

The conditions in the microcosms were kept unmodified for 5 days to allow plant acclimation. After that time, no symptoms of stress were apparent and photosynthetic parameters (see below) were close to those found in the field (A. Gera, unpublished results). Moreover, the redox stratification observed in the field (2–3 cm light-grey coloured sediment over a dark layer) was recovered after 2–3 days. After the acclimation period, adequate amounts of salt were added so as to raise the salinity of the aquaria (except controls). Such addition lasted for one hour; this was slow enough to prevent (or at least attenuate) osmotic shock, but compatible with increments in salinity associated with a brine discharge (Torquemada and Lizaso, 2005). Final salinities were as follows: 3 controls at 37.2 psu; 3 at 44 psu; 3 at 54 psu; and 3 at 62 psu. In order to avoid experimental bias and non-demonic intrusion, treatments were completely randomized and interspersed (Hurlbert, 1984). The plants were maintained under these conditions for 17 days, during which leaf quantum efficiency was measured periodically. At the end of the period, the plants were harvested and analysed as described below.

2.2. Plant response measurements

The quantum efficiency of photosystem II or *Fv*/*Fm* ratio (Beer et al., 1998; Durako and Kunzelman, 2002) was measured as an expression of photosynthesis functionality (Ralph, 1999) the day before the addition of salt and at 1, 2, 6, 9 and 16 days after that, on dark-adapted (10 min) leaves, using a diving PAM (Pulse Amplitude Modulation; Walz, Germany). Five quantum efficiency measurements were taken per aquarium (i.e. we determined the *Fv*/*Fm* ratio from five leaves, each of them from a different shoot). We always determined *Fv*/*Fm* ratio on the middle portion of rank 2 blades (second youngest leaves) as those have been shown to be the most representative tissues for assessing quantum efficiency with the lowest within-shoot variability (Durako and Kunzelman, 2002).

Leaf growth was determined using a modified Zieman's method (Zieman, 1974; Pérez and Romero, 1994). We marked three shoots in each aquarium immediately before salt addition. These shoots were separated from the rest at the end of the experiment, sorted into new and old tissue and dried at 70 °C for 48 h (until constant weight was reached). Subsequently, the biomass (as g DW m^{-2}) of each fraction was determined to calculate production as g DW $shoot^{-1}$ day^{-1}.

Leaf necrosis has been shown to be associated with seagrass deterioration (Romero et al., 2007) and specifically with salinity stress (Gacia et al., 2007). To quantitatively assess the incidence of necrosis in leaves, we established three categories, namely: "green" (with no evident necrosis spots), "spotted" (with evident necrosis spots, but with less than the 75% of the surface necrotic) and "black" (with more than the 75% of the surface necrotic). We counted the number of leaves in each category and we expressed necrosis incidence as a percentage relative to the total number of leaves present in each aquarium. As no leaf abscission took place during the experiment, we considered as dead shoots those with all leaves belonging to the "black" (fully necrotic) category. Shoot mortality was hence expressed as the percentage of the number of dead shoots relative to the total number of shoots per aquarium.

At the end of experiment, all the shoots were harvested, counted and separated into leaves, rhizomes and roots, and dried at 70 °C for 48 h (until constant weight was reached). Subsequently, the biomass (as g DW m^{-2}) of each plant fraction was determined.

2.3. Statistical analysis

We considered "aquarium" the experimental unit, which was threefold replicated for each treatment. Even if all treatments were placed in the same chamber, this chamber was sufficiently heterogeneous (see above) to avoid pseudoreplication (Hurlbert, 1984). Similar designs have been used in previous experiments using mesocosms (e.g. Torquemada and Lizaso, 2005; Mascaró et al., 2009). PAM fluorimetry data were analysed using repeated measures ANOVA (RMANOVA) design considering one between-subjects factor (salinity, with four levels: control [37.2], 44, 54 and 62 psu) and one within-subjects factor (i.e. time, with six levels, the six events of measurements). Our data satisfied sphericity (Mauchly test $p > 0.05$), normality and homogeneity of variances.

The rest of the variables were evaluated using a one-way ANOVA considering "salinity" a fixed factor with four levels (control [37.2], 44, 54 and 62 psu). Before analysis, the data were tested for normality and homoscedasticity using the Shapiro–Wilk's and Barlett's test respectively. Shoot mortality was analysed with a non-parametric (Kruskal–Wallis) test, as this variable did not follow normality. Normality was fulfilled in the rest of the cases; but when the homoscedasticity assumption was not satisfied, variables were square root transformed.

Whenever an ANOVA was significant for any factor, a multiple range contrast test was applied (Fisher's LSD) to determine specific treatment differences. Calculations were performed using Statistica ver. 6.0 software.

3. Results

At the beginning of the experiment plants from all the aquaria presented similar values of leaf quantum efficiency (*Fv*/*Fm*). The day after salt addition, *Fv*/*Fm* dropped to 56% of the initial values only in plants in the high salinity (62 psu) aquaria and then their values remained unchanged until the end of the experiment. No significant changes in *Fv*/*Fm* were found in the control, 44 and 54 psu treatments (Fig. 1, Table 1).

Leaf growth rates were lower in the 62 psu and 54 psu aquaria than in the rest (approximately 50% and 80% of that of controls), while they remained similar to the controls in the 44 psu aquaria (Fig. 2). However, due to the high variability encountered, overall differences were only marginally significant ($p<0.1$; Table 1). Post-hoc analyses (LSD) confirmed leaf growth rates in the 62 psu aquaria as significantly lower than those in the 44 psu aquaria and controls.

Salinity increased leaf necrosis incidence, which almost doubled in the 62 psu and 54 psu aquaria, relative to controls, while it remained unaltered in the 44 psu aquaria (Fig. 3). Shoot mortality was only observed in the 54 and 62 psu treatments, with values of 9% and 15%, respectively (Fig. 4). No shoot mortality was observed in the 44 psu salinity aquaria or in controls.

Rhizome and leaf biomass were not significantly affected by salinity increase, despite the existence of a trend towards reduction at high salinities (Fig. 5a). In contrast, biomass of the root fraction was significantly affected by salinity; it was lowest (LSD post-hoc test) in the 62 psu treatment, whose root biomass was roughly one third of that of controls (Fig. 5b).

Table 1
Summary of the different ANOVA analyses performed. *P*-values correspond to those provided by an *F*-test. *df*, degrees of freedom. n.s., non-significant.

Variable	Effects	*df*	% variance	*p*-value
Photosynthetic efficiency (between-subjects)	Salinity	3	84.53	0.001
	Error	8	15.47	
Photosynthetic efficiency (within-subjects)	Time	5	51.25	0.000
	Time × Salinity	15	34.68	0.000
	Error	40	14.07	
Leaf growth	Salinity	3	54.53	0.084
	Error	8	45.47	
%Black leaves	Salinity	3	68.59	0.021
	Error	8	31.41	
%Spotted leaves	Salinity	3	67.57	0.023
	Error	8	32.43	
%Green leaves	Salinity	3	64.38	0.033
	Error	8	35.62	
%Dead shoots	Salinity	Non-parametric (Kruskal–Wallis)		0.021
	Error	8	32.43	
Total leaf biomass	Salinity	3	–	n.s.
	Error	8	100	
Green leaves biomass	Salinity	3	–	n.s.
	Error	8	100	
Root biomass	Salinity	3	73.51	0.011
	Error	8	26.49	
Rhizome biomass	Salinity	3	–	n.s.
	Error	8	100	

4. Discussion

Our results show that, at least in the short term, *C. nodosa* is able to tolerate sustained and moderate salinity increases, up to 44 psu, without any apparent damage. However, it loses vitality as salinity increases further (54 psu) and suffers substantial mortality at values of 62 psu. At a salinity of 54 psu, the plant showed diverse symptoms of alteration, including an increase of necrosis incidence, which affected 43% of leaves (less than 20% in controls) and a significant mortality (9% of shoots died at the end of the 17-day period, no mortality in controls). However, photosynthesis remained unaltered (at the same level as in control aquaria). Under severe hypersaline conditions (62 psu) not only were the *Fv*/*Fm* ratio and root biomass drastically reduced (to about one half and one third of that of controls, respectively) but also leaf necrosis peaked, causing the death of 15% of the shoots during the study period. It seems likely that under continued exposure to 62 psu all the shoots would have died within a few weeks.

These results are consistent with a number of other studies, which found similar responses in seagrasses exposed to hypersaline conditions (Walker and McComb, 1990; Kamermans et al., 1999; Murphy et al., 2003; Torquemada and Lizaso, 2005; Torquemada and Lizaso, 2006; Gacia et al., 2007; Koch et al., 2007a,b). However, the available evidence suggests that timing, salinity thresholds and the magnitude of the response vary greatly between species. In effect, previous work aimed at assessing the behaviour of seagrass under hypersaline conditions has shown responses that range from considerable sensitivity (e.g. in the Mediterranean endemics *P. oceanica*; Torquemada and Lizaso, 2005; Gacia et al., 2007) through moderate sensitivity (e.g. *Zostera marina* and *Zostera noltii*; van Katwijk et al., 1999; Torquemada and Lizaso, 2006) to high tolerance (to as much as 60 psu for *Thalassia testudinum* and 65 psu for *Halodule wrightii*; Koch et al., 2007b).

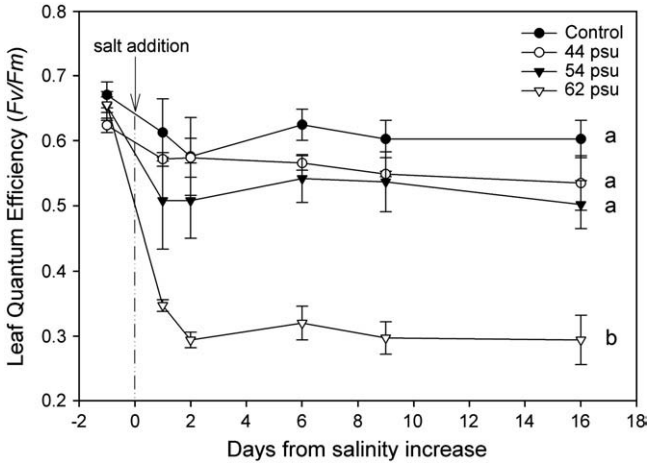

Fig. 1. *Cymodocea nodosa* dark-adapted leaf quantum efficiency evolution during the experimental period (mean ± SE, $n=3$). Lines labelled with the same lower case letter do not differ significantly according to Fisher's LSD post-hoc test.

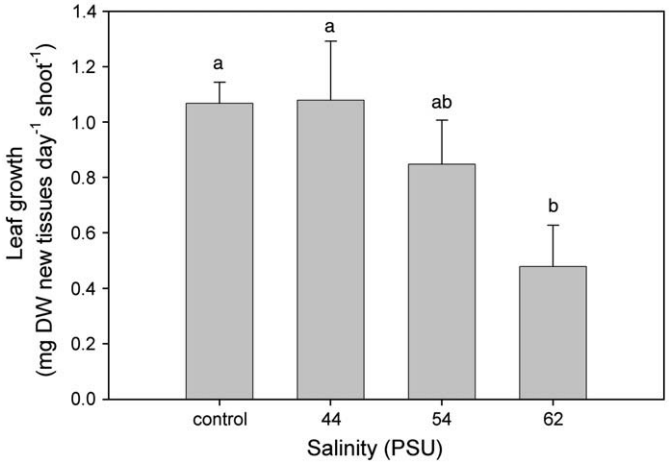

Fig. 2. *Cymodocea nodosa* mean leaf growth in each salinity treatment (mean ± SE, $n=3$). Bars labelled with the same lower case letter do not differ significantly according to Fisher's LSD post-hoc test.

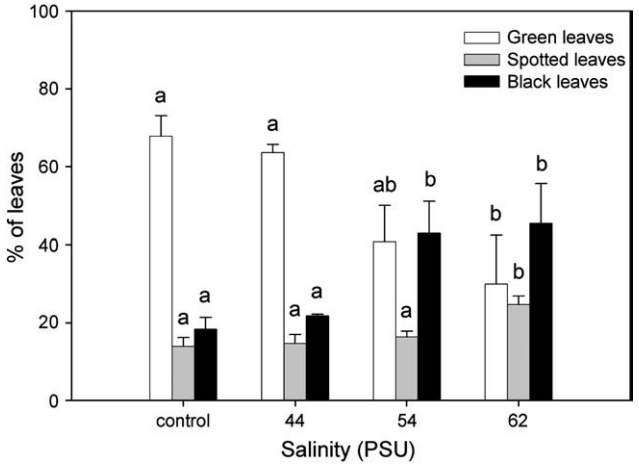

Fig. 3. Leaf necrosis incidence represented by the mean percentages of green leaves (with no sign of necrosis), spotted leaves (<75% of surface necrotic), and black leaves (>75% of surface necrotic) in the different treatments (mean ± SE, $n = 3$). Bars labelled with the same lower case letter do not differ significantly according to Fisher's LSD post-hoc test.

Our results suggest two main issues for future research in the field. Firstly, while our general knowledge of the response of seagrasses to increased salt concentration has considerably increased over the last decade, our comprehension of the basic physiological mechanisms involved remains incomplete (Touchette, 2007). Salinity fluctuations can importantly alter plant biochemical and physiological processes, which in turn can influence plant metabolism, growth, development and reproduction (McMillan and Moseley, 1967; Walker and McComb, 1990; Vermaat et al., 2000; Torquemada et al., 2005a). Specifically, saline stress can decrease photosynthetic capacity in seagrasses (Biebl and McRoy, 1971; Ralph, 1998; Torquemada et al., 2005a) probably via a decline in chlorophyll content (Baek et al., 2005; Karimi et al., 2005), a change in chloroplast structure or inhibition of the activity of key photosynthetic enzymes (Touchette, 2007). Our results seem to support these findings to a certain point, as *C. nodosa* shoots from the most saline treatment showed a steep decline in photosynthetic activity as indicated by a decrease in the *Fv*/*Fm* ratio (Kamermans et al., 1999; Murphy et al., 2003; Koch et al., 2007a). Nevertheless, the plants maintained a rather high *Fv*/*Fm* ratio up to a salinity of 54 psu. This suggests that the cause of the symptoms observed (low shoot growth and significant incidence of leaf necrosis) was not the loss of integrity of the photosynthetic apparatus, or at least not at this salinity. Therefore, other causes should be invoked to account for the loss of vitality at 54 psu. Among them, the alterations in turgor pressure (related to the elasticity of the cell walls, ε of plant cells due to water fluxes in the direction of the osmotic gradient can play a key role. According to Touchette (2007) plants with relatively low ε (i.e. flexible cell walls) are more tolerant to short-term fluctuations in salinity, as walls can expand or contract until they achieve osmotic equilibrium with their environment. Low ε is particularly useful during hyperosmotic conditions since it decreases the likelihood of plasmolysis during water efflux.

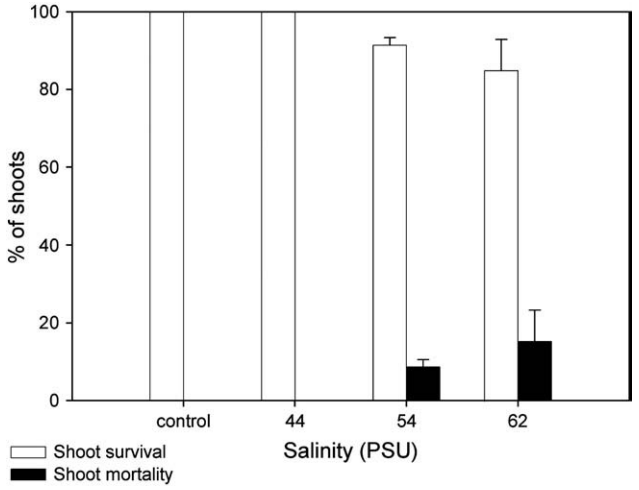

Fig. 4. Percentage of shoot mortality and shoot survival in each salinity (mean ± SE, $n = 3$).

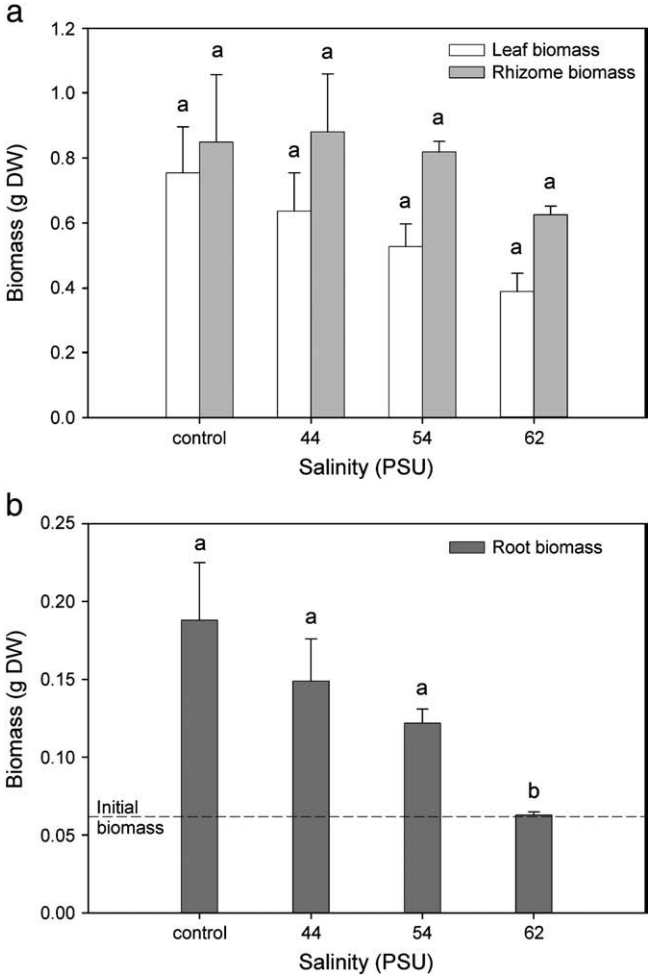

Fig. 5. *Cymodocea nodosa* leaf, rhizome (a) and root (b) biomass at the end of the experiment in each fraction across the range of salinities tested (mean ± SE, $n = 3$). The dashed line in (b) indicates the initial mean root biomass ($n = 15$). Bars labelled with the same lower case letter do not differ significantly according to Fisher's LSD post-hoc test.

The second issue deserving attention is the effect of high salinity on the belowground compartment, which, as far as we are aware, has received little attention so far. However, according to our results, roots seem to be very sensitive to salinity increases. In agreement with the natural pattern at that time of year (Pérez and Romero, 1994), root growth, as indicated by root biomass increase, took place in the control, 44 and 54 psu aquaria (relative to initial values). However no such growth was observed in the 62 psu treatment, as final values were very close to initial ones. A failure to develop underground structure can have deleterious effects on plant vitality, especially in cases where root uptake is the main nutrient source for plant growth (Romero et al., 2006). Although the root affectation could also have been caused by metabolic damage to the leaves, root affectation is especially concerning in the light of previous work that reports a low turnover rate, and, hence, a longer residence time of the brine in the