



Salt application as an effective measure to control ruderal invaders threatening endangered halophytic plant species

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Balearic islands; Conservation management; Germination success; Halophyte vegetation; Mallorca; Restoration; Salinity stress; Salt application regime; Salt marsh invasion; Sea lavender

Nomenclature

Blarney & Grey-Wilson 1998

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Abstract

Questions: How does salinity affect germination, seedling performance and survival of the critically endangered *Limonium barceloi*, compared to ruderal species invading its salt marsh habitat? Is salt application an effective management tool for controlling invaders and favouring endangered halophytes?

Location: Ses Fontanelles, Mallorca, Balearic Islands, Spain.

Methods: The germination of seeds of *L. barceloi* and nine ruderal species was tested in five salinity levels (0–37 g·L⁻¹), and also in freshwater after a pre-treatment with seawater. The ruderal study species were: *Asphodelus fistulosus*, *Avena barbata*, *Geranium molle*, *Hypochoeris achyrophorus*, *Oryzopsis miliacea*, *Plantago coronopus*, *Reichardia picroides*, *Sonchus asper* and *Sonchus tenerrimus*. Two salt concentration levels (18 and 37 g·L⁻¹) were applied twice monthly, weekly or twice weekly to examine the effect of salt irrigation schemes on the performance and seedling survival of *L. barceloi* compared to two of the principal species invading these salt marshes, *S. tenerrimus* and *A. fistulosus*.

Results: Germination of *L. barceloi* and most ruderal species was highest at low salinity (0–8 g·L⁻¹). Seeds of *L. barceloi*, *A. fistulosus* and *S. tenerrimus* were also able to germinate at the highest salinity level, in contrast to the other species. Germination in freshwater after seawater pre-treatment was highest in *L. barceloi* (72 ± 6%). Seedling performance and survival of *A. fistulosus* and *S. tenerrimus* decreased with frequency and concentration of the salt treatment. An application of 37 g·L⁻¹ salt twice a week reduced seedling survival of *A. fistulosus* and *S. tenerrimus* by 83.3 and 91.6%, respectively. In contrast, 100% of the *L. barceloi* seedlings survived and showed similar performance among treatments.

Conclusions: *Limonium barceloi* had higher seedling growth and survival than ruderal invaders when salinity levels were high (37 g·L⁻¹). Nevertheless, at lower salinity, ruderal species may germinate and grow better and thus salinity level changes may represent a threat to the survival of this critically endangered species. Although further field testing is required, our experiments suggest that salt application could be an effective measure to protect *L. barceloi* and other endangered halophytic plant species from less salt-tolerant invaders.

Introduction

Salt marshes are dynamic ecosystems with frequent and significant fluctuations in salinity and water level (Pashkoff 1985; Adam 1990; Pennings & Bertness 2001), which can increase biodiversity by supporting rare species. Located on lowland coastal fringes, inputs of salt via sea-water originate from salt spray and tidal and subterranean

marine flows. These combine with freshwater run-off to produce hydrological regimes (Mendelssohn & Morris 1999; Mitsch & Gosselink 2000) that vary annually and inter-annually and can result in unique communities (Gedan et al. 2009). These communities are threatened by habitat destruction from urban development and hydrological modification (Adam 2002; Gedan et al. 2009). Wetland systems are very susceptible to changes in quantity

and quality of the water supply due to changes in the water catchment, freshwater inflows, sewage spills and sea level rise, which can lead to a modification of substrate salinity (Adam 1990; Erwin 2009). As salinity is the principal factor driving patterns of plant species persistence and community composition in salt marshes (e.g. Oosting & Billings 1942; Kachi & Hirose 1979; Vince & Snow 1984; Bertness & Pennings 2000.; Pennings et al. 2005; Griffiths 2006; Álvarez-Rogel et al. 2007; Watson & Byrne 2009) changes in salinity could further threaten endangered species. A reduction in substrate salinity due to increased fresh water inflows is a particular problem (Erwin 2009), because it may promote inter-specific competition between halophytic salt marsh specialists and non-halophyte species intruding into the habitat (Callaway et al. 1990; Callaway 1994; Callaway & Sabraw 1994). Changes in competitive outcome have been observed in California, where *Polypogon monspeliensis* displaces *Salicornia virginica* (Callaway & Zedler 1997; Kuhn & Zedler 1997), and in Western Australia where *Typha orientalis* replaces *Juncus kraussii* (Zedler et al. 1990). If such a reduction in salinity is prolonged, it may cause changes in community structure and local extinctions of narrowly adapted halophytes (Bakkenes et al. 2002; Peterson et al. 2002).

Hydrologic changes impacting salinity dynamics are of particular conservation concern if they involve endangered salt marsh species. Such a species is the critically endangered sea lavender *Limonium barceloi* Gil & Llorens (Plumbaginaceae) (Gil & Llorens 1991; Rosselló & Sáez 2004) on the island of Mallorca, Spain. The last remaining *in situ* population dramatically decreased in number following accidental freshwater flooding of the site. After the flooding event, a series of opportunistic ruderal species invaded the areas previously inhabited by *L. barceloi* and other salt marsh specialists (Khan & Traveset 2009a). On a separate *ex-situ* study site, adjacent to Ses Fontanelles, managers have controlled ruderal species in the past through irrigation with Mediterranean seawater (37 g·L⁻¹; Franquesa 2009). Managers currently suggest salt-water irrigations of the *in-situ* site to assist in species recovery in the areas affected by freshwater inundation (Franquesa 2009).

The aim of this study was to inform management conservation actions for the critically endangered *L. barceloi*, by examining the germination and seedling establishment of *L. barceloi* and nine ruderal species subjected to various levels of salinity. In a series of controlled experiments, we examined (1) the effects of various salinity levels on the percentage and rate of germination of *L. barceloi* compared to ruderal species; (2) the potential of the seeds of *L. barceloi* compared to the other study species to recover from a pre-treatment with high salinity; (3) seedling performance and proportion of surviving seedlings of two ruderal species and *L. barceloi* at different salt concentration

and application frequencies; and (4) the applicability of a salt-water irrigation regime to control ruderal species. The specific hypotheses tested were that *L. barceloi* would show (1) higher tolerance over a range of salt conditions at the germination stage than the other species, (2) increased capacity of seeds to germinate after a pre-treatment with seawater, (3) improved seedling performance and survival under high concentrations and frequencies of salt application compared to the ruderal species, and (4) that salt application could be an effective measure to control ruderal invaders in salt marshes with freshwater intrusion.

Our study provides the first analysis of salt tolerance at the germination and seedling development stage for this critically endangered species and for nine ruderal species present at the Ses Fontanelles salt marsh. This study is an important first step towards making practical recommendations for using salt applications to control ruderal species in salt marshes. The work is relevant to managers of endangered species of worldwide salt marshes threatened by freshwater intrusion.

Methods

Study species

Limonium barceloi is an herbaceous perennial or low-growing shrub endemic to the island of Mallorca and currently restricted to a single salt marsh fragment, Ses Fontanelles (39°32'05.92" N, 2°43'41.60" E), in the Bay of Palma, Spain. Following the rupture of a water pipe on the site in 2009, and the subsequent flooding of the area with freshwater, the population was reduced from 3000 to 350 individuals (Khan & Traveset 2009a). The freshwater intrusion, which lasted for 8 d, also resulted in a pronounced decline in substrate salinity from 33.8 dS·m⁻¹ (25 °C, 30 cm depth) 6 mo before freshwater inundation, to 6.44 dS·m⁻¹ (25 °C, 30 cm depth) 6 mo after freshwater inundation (Franquesa 2009; Khan & Traveset 2009a). *L. barceloi* is associated with other halophytic vegetation in this salt marsh remnant (Gil & Llorens 1991). Following the freshwater intrusion, a series of opportunistic ruderal species invaded the salt marsh. Nine of these species were selected for study. These species are common native ruderals that grew abundantly in close proximity to *L. barceloi* (Khan & Traveset 2009a), namely: *Asphodelus fistulosus* L. (Liliaceae, perennial (p)), *Avena barbata* Pott ex Link (Gramineae, annual (a)), *Geranium molle* L. (Geraniaceae, a), *Hypochoeris achyrophorus* L. (Compositae, a), *Oryzopsis miliaceae* L. (Gramineae, p), *Plantago coronopus* L. (Plantaginaceae biennial (b)/p), *Reichardia picroides* L. (Compositae, a/p), *Sonchus asper* L. (Compositae, a) and *Sonchus tenerrimus* L. (Compositae, a/b/p). Nomenclature follows Blamey & Grey-Wilson 1998.

Seed material

Seeds of *L. barceloi* were collected in August 2009 from 60 individuals located in Ses Fontanelles. Seeds of the other species were provided from the Botanical Gardens Conservation International Network (BGCI) based in Kew Gardens, London, UK. Seeds from the BGCI network were collected and stored under standard guidelines to ensure homogeneity of sample, condition and viability. Upon receipt, all seed samples were maintained at 4 °C in dry conditions until the experiment started. Only viable seeds were used for the experiments. Viability of *L. barceloi* seeds was assessed based on reference values of seed weight of viable seeds (viable seeds are heavier than non-viable ones; Bibiloni 2000).

Germination experiment

The germination experiment was conducted using ten species. In addition to *L. barceloi*, the nine ruderal species (see Study species) described above were used. We used a total of 300 seeds per species in the germination experiment. Twenty seeds of each species were placed separately in 85-mm diameter Petri dishes containing filter paper discs and 8 ml of the test solution. Seeds in each Petri dish were randomly assigned to five salinity treatments: 0 g·L⁻¹ (control), 4 g·L⁻¹, 8 g·L⁻¹, 16 g·L⁻¹ and 37 g·L⁻¹. Three replicates of each species were used for each treatment, thus a total of 15 Petri dishes were used for each species. Salt solutions were prepared using distilled water and Mediterranean seawater, which has a salinity of 37 g·L⁻¹.

Petri dishes with seeds were placed in a climate chamber with a 12-h photoperiod at 18 °C. These conditions were chosen to reflect temperature and photoperiod in March (1998–2008 inclusive: mean maximum temperature 18.2 °C ± 1.19 (Amengual et al. 2011); average day length 717.1 min ± 0.83 (Observatorio Astronómico Nacional 1998–2008). March is the period peak germination at Ses Fontanelles (Bibiloni 2002). Seeds were checked every 2 d, and solutions replenished when necessary. Every 4 d for 4 wk, germinated seeds were counted and removed; a seed was considered to have germinated if the radicle had emerged (Copeland & McDonald 1995). We terminated the experiment after 4 wk.

Germination after salt treatment experiment

To determine potential seed germination after the salinity treatments in the germination experiment, all seeds that had not germinated in the germination experiment with 37 g·L⁻¹ solutions were rinsed thoroughly in distilled water and placed into Petri dishes on filter paper with

distilled water. The germination of these seeds was monitored for 7 d to determine viability.

Experimental testing of seedling performance and survival under salt application schemes

The effects of irrigation with different salt concentrations at varying frequencies were tested on the performance and survival of *L. barceloi*, *A. fistulosus* and *S. tenerrimus* seedlings in a greenhouse experiment in June 2010. The two latter species were chosen because they are among the most abundant species at the site and also readily germinated after salinity treatments in the germination experiment.

Seedlings of these three species were chosen at random from the previous germination experiment and transferred into 500-cm³ pots lined with small clay pellets and filled with a mix of 60% peat, 20% coconut fibre and 20% fine sand (Jardí Botànic de Sóller 2007). Seedlings were grown for 6 wk and then randomly assigned to one of seven treatments in a full-factorial design with two factors: irrigation frequency (twice a week, weekly or fortnightly) and salinity concentration at irrigation (0 g·L⁻¹: control, 18 g·L⁻¹ or 37 g·L⁻¹). These concentrations, i.e. ca. 50% dilution of Mediterranean seawater (18 g·L⁻¹) or 100% seawater (37 g·L⁻¹) were chosen as concentrations that could be practically applied in the field by conservation managers.

The experiment was conducted for 4 wk, beginning on 2 June 2010. We used 36 seedlings of each species in the control treatment (one seedling per pot) and 12 seedlings of each of the three species (one seedling per pot) for all other treatments. The saline and control solutions were applied via a mister and at a standardized volume of 50 ml. This form of salt application reflects the most feasible approach for conservation managers in the field. All individuals were watered with distilled water once per day to prevent water stress, which might reduce osmotic stress under high salinity but was unlikely to be significant. We did not measure soil salinities because we were primarily interested in the overall effects of different salt application regimes rather than in knowing the precise salinity levels experienced by the plants.

Three different measurements were taken of each plant on day 1, 7, 14, 21 and 28: total number of leaves (leaf number), length of the longest leaf (leaf length) and plant height. Biomass was not assessed so as not to sacrifice individuals of the endangered *L. barceloi*. Post-experiment seedlings were given to managers to augment the existing *L. barceloi* *in situ* population.

Data analysis

An ANOVA was used to analyse the response variables. To test whether the effect of salinity level on germination of

seeds (number of germinated seeds at the end of the experiment) differed among the study species, ANOVA (linear model) with species, salinity treatment (factor with five salinity levels) and the interaction of species \times salinity treatment was performed. Since the sown number of seeds was identical for all species, we used the number of germinated seeds as the most direct response variable instead of germination percentage. As the species \times salinity treatment interaction indicated significantly different responses of the different species to the salinity treatments, separate ANOVAs and multiple comparisons of means using Tukey's HSD *post-hoc* tests were performed for each salinity level.

The same linear models (ANOVAs) described above for seed germination were used to analyse variation in the response variable mean time to germination (MTG). MTG was calculated following Brenchley & Probert (1998) using the equation:

$$\text{MTG} = \frac{\sum(n \times d)}{N}$$

where n is number of seeds germinated between scoring intervals; d is incubation period in days at that time point, and N is total number of seeds germinated in the treatment. Replicates (Petri dishes) with no germinated seeds were excluded from the analysis of MTG. A one-way ANOVA (generalized linear model using a quasi-binomial error structure and a logit link function) was used to analyse the proportion of germinated seeds after seawater pre-treatment (Warton & Hui 2011; Crawley 2007). A quasi-binomial error structure was used to account for overdispersion in the data (Crawley 2007). Tukey's HSD *post-hoc* tests were performed to test for differences among species.

To investigate whether seedling performance (response variables: numbers of leaves, length of longest leaf and plant height at the end of the experiment) of the study species *L. barceloi*, *A. fistulosus* and *S. tenerrimus* responded differently to the salt application treatments, ANOVAs (linear model) with the explanatory variables species, salt application treatment (factor with seven levels) and the interaction between species \times treatment were performed. ANOVA (generalized linear model using a quasi-binomial error structure and a logit link function) was used to analyse variation in seedling survival (i.e. proportion of individuals surviving at the end of the experiment). A quasi-binomial error structure and F -tests were used to account for overdispersion in the data (Crawley 2007). For all response variables (number of leaves, longest leaf length, plant height and seedling survival), significant species \times salt application treatment interactions indicated different responses of species to the treatments. Therefore, separate ANOVAs for each species and multiple comparisons of means using

Tukey's HSD *post-hoc* tests were performed to detect treatment differences for each response variable.

All statistical analyses were done using the software package *R* (R Foundation for Statistical Computing, Vienna, AT). Model fit and the assumptions of ANOVA were assessed by plotting the residuals against the predicted values. All data analysed with linear models met the assumptions of normality and homoscedasticity and were therefore not transformed. Arithmetic means (± 1 SE) are reported.

Results

Salinity and germination

The study species differed in seed germination at different salinity levels (significant species \times salinity treatment interaction: $F_{36,100} = 14.53$, $P < 0.001$; Fig. 1). Subsequent separate analyses of each study species showed that germination was significantly affected by the salinity treatment (all $P < 0.001$; Fig. 1), with the exception of *O. milicea* ($F_{4,10} = 2.25$, $P < 0.136$), which had low germination at all salinity levels (Fig. 1). Germination of the other species was similar in the non-saline control treatment and at low salinity (4 g·L⁻¹ and/or 8 g·L⁻¹), except for *G. molle*, which showed reduced germination at low salinity compared to the non-saline control (Fig. 1). In the highest salinity (37 g·L⁻¹), only three species germinated: *L. barceloi*, *S. tenerrimus* and *A. fistulosus* (Fig. 1; germination = 30, 10 and 5%, respectively). These three species were also the only study species to germinate in all treatments. Germination at intermediate salinity (16 g·L⁻¹) was significantly lower than at low salinity in all species except *A. barbata*, and was significantly higher than at the highest salinity for *L. barceloi*, *S. asper*, *P. coronopus*, *A. barbata* and *H. achyrophorus* (Fig. 1).

The study species differed in germination rate after pre-treatment in seawater ($F_{9,20} = 37.44$, $P < 0.001$). The germination rate of *L. barceloi* after pre-treatment with seawater (71.7 \pm 6.0%) was significantly higher than that of *S. asper* (43.3 \pm 4.4%), *H. achyrophorus* (28.3 \pm 4.4%), *O. milicea* (18.3 \pm 1.7%), *A. barbata* (1.7 \pm 1.7%), *G. molle* (0.0 \pm 0.0%) and *R. picroides* (0.0 \pm 0.0%) (all $P < 0.05$), but not significantly different to the germination rate of *P. coronopus* (68.3 \pm 1.7%), *A. fistulosus* (58.3 \pm 7.3%), *S. tenerrimus* (55.0 \pm 8.7%).

Mean germination time (MTG) of the study species differed by salinity level (significant species \times salinity treatment interaction: $F_{26,76} = 5.06$, $P < 0.001$; Fig. 2). Separate analysis of each study species showed a significant effect of salinity on MTG in most species (all $P \leq 0.044$; Fig. 2) except for *A. barbata*, *R. picroides* and *O. milicea* ($P \geq 0.070$; Fig. 2). In *L. barceloi*, *S. asper*, *S. tenerrimus*, *P. coronopus*, *G. molle* and *H. achyrophorus*, germination

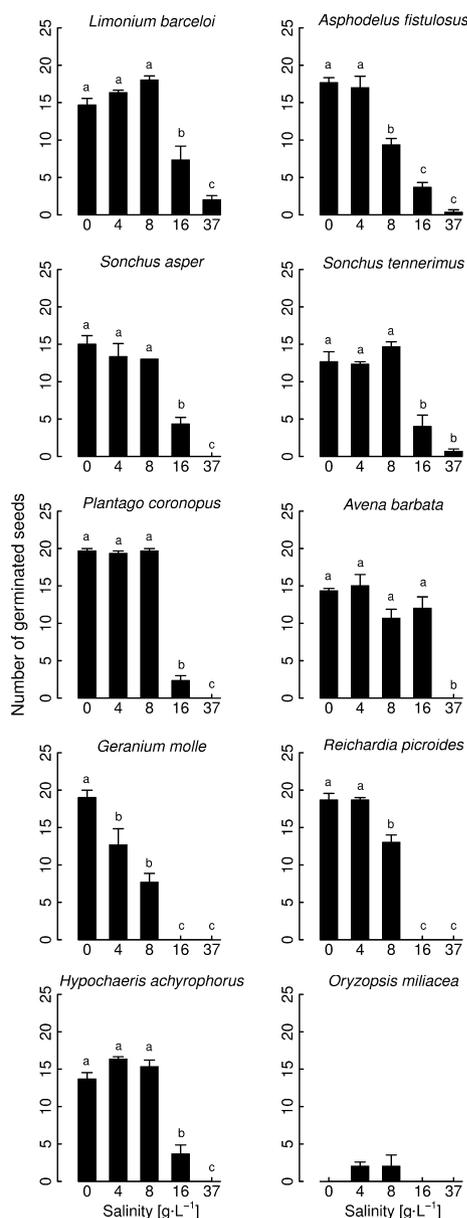


Fig. 1. Mean (± 1 SE) number of germinated seeds by species after 28 d under salt concentrations from 0 to 37 g·L⁻¹. Twenty seeds were sown in each of three Petri dishes for each species. Different letters indicate significant differences among salinity levels ($P \leq 0.05$; no letters indicate no significant differences among treatments for a species).

was slower at high salinity compared to lower salinity or the non-saline control (Fig. 2).

Seedling performance and survival

The growth performance of seedlings of *L. barceloi*, *S. tennerimus* and *A. fistulosus* differed by species and salinity level, i.e. species \times treatment interaction for the number of leaves ($F_{12,223} = 6.37$, $P < 0.001$), length of the longest leaf

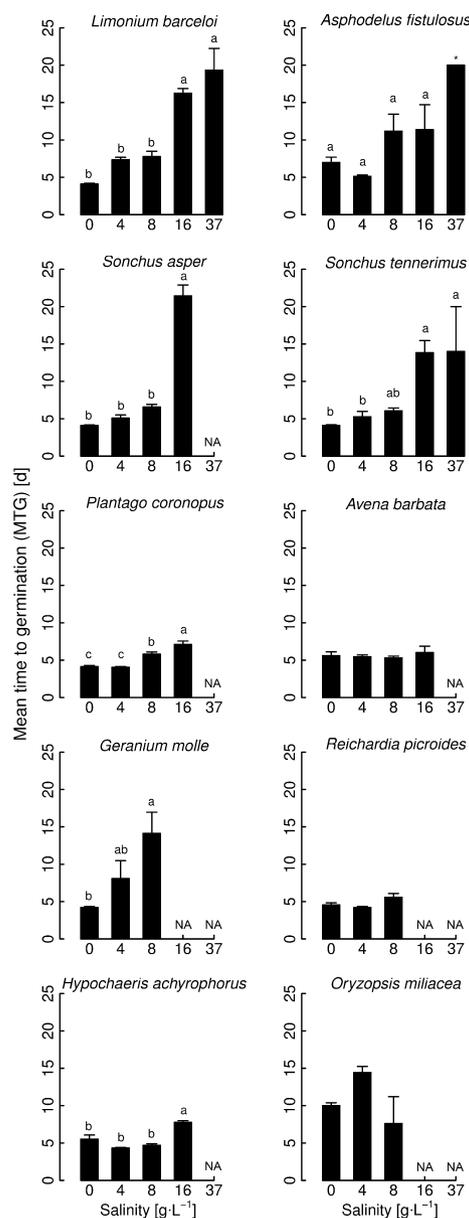


Fig. 2. Mean (± 1 SE) time to germination (MTG) of *L. barceloi* and nine ruderal, potential competitor species under salt concentrations from 0 to 37 g·L⁻¹. Different letters indicate significant differences among salinity levels ($P \leq 0.05$). If no seeds germinated, MTG could not be calculated and these seeds were excluded from the *post-hoc* analysis. * indicates a single observation for a treatment, which was not included from treatment comparisons using Tukey *post-hoc* tests. Treatments labelled 'na' had no seed germination and were also excluded from the analysis.

($F_{12,223} = 10.81$, $P < 0.001$) and height ($F_{12,223} = 12.13$, $P < 0.001$) (Fig. 3).

Separate ANOVAs and Tukey's *post-hoc* tests for each species revealed that the number of leaves of seedlings of *L. barceloi* did not differ in any salt application treatment (Fig. 3a). The length of the longest leaf and height of

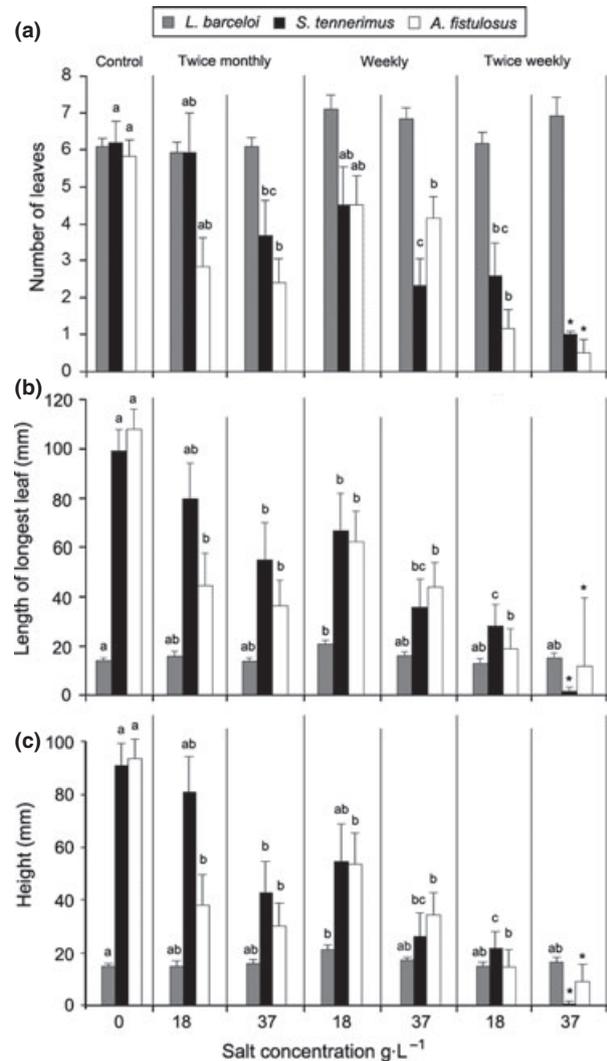


Fig. 3. Mean \pm SE for (a) number of leaves, (b) length of the longest leaf and (c) height (cm) of *L. barceloi*, *S. tenerrimus* and *A. fistulosus* after 28 d under six salt application regimes, differing in application frequency (twice a month, weekly, twice a week) and salt concentration per application (18 and 37 g·L⁻¹) and a control treatment (0 g·L⁻¹). Different letters indicate significant differences among salinity levels ($P \leq 0.05$). Different fonts indicate different species. * indicates that few seedlings (≤ 2) survived in a treatment; these were therefore excluded from the analysis.

seedlings of *L. barceloi* increased only after weekly application of 18 g·L⁻¹ saline solution compared to the non-saline control (Fig. 3b,c). In contrast, there were significant treatment effects on the seedling performance for *S. tenerrimus* (number of leaves: $F_{6,58} = 13.45$, $P < 0.001$; longest leaf length: $F_{6,58} = 16.93$, $P < 0.001$; height: $F_{6,58} = 22.85$, $P < 0.001$) and *A. fistulosus* (number of leaves: $F_{6,64} = 5.39$, $P < 0.001$; longest leaf length: $F_{6,64} = 9.70$, $P < 0.001$; height: $F_{6,64} = 8.90$, $P < 0.001$) (Fig. 3). Significant reductions in these seedling performance indicators in *S. tenerrimus* and *A. fistulosus* compared to the non-saline control

treatment were typically measured after 37 g·L⁻¹ salt solution twice a month, with higher salt application frequencies resulting in significantly lower performance of seedlings of *S. tenerrimus*, but not of *A. fistulosus* (Fig. 3). A weekly application of 37 g·L⁻¹ saline solution reduced seedling performance of *S. tenerrimus* by 62.5% (number of leaves), 70.0% (height) and 63.6% (length of longest leaf), while 37 g·L⁻¹ saline solution twice a week resulted in a reduction of 83.9% (number of leaves), 99.0% (plant height) and 98.4% (length of longest leaf) (Fig. 3). In *A. fistulosus*, a weekly application of 37 g·L⁻¹ saline solution reduced seedling performance by 28.8% (number of leaves), 63.4% (plant height) and 59.5% (length of longest leaf), and 37 g·L⁻¹ saline solution twice a week resulted in a reduction of 91.4% (number of leaves), 90.3% (plant height) and 89.0% (length of longest leaf) (Fig. 3).

The salt application treatments had different effects on the proportion of surviving seedlings of three species (significant species \times treatment interaction: $F_{12,303} = 3.03$, $P = 0.001$) (Fig. 4). A significant treatment effect was found for the proportion of surviving seedlings of *S. tenerrimus* ($F_{6,101} = 3.94$, $P = 0.001$) and *A. fistulosus* ($F_{6,101} = 6.90$, $P = 0.001$), reflecting the pronounced decrease in seedling survival of these species, especially at the highest salt application frequency (twice a week) and salt concentration (37 g·L⁻¹; Fig. 4). Application of 37 g·L⁻¹ saline solution reduced seedling survival of *S. tenerrimus* by 50.0% when applied weekly, and by 91.6% when applied twice a week (Fig. 4); similarly, reductions in seedling survival of *A. fistulosus* were 33.3% and 83.3%, respectively. In contrast, all seedlings of *L. barceloi* survived until the end of the experiment, irrespective of the salt application treatment (Fig. 4).

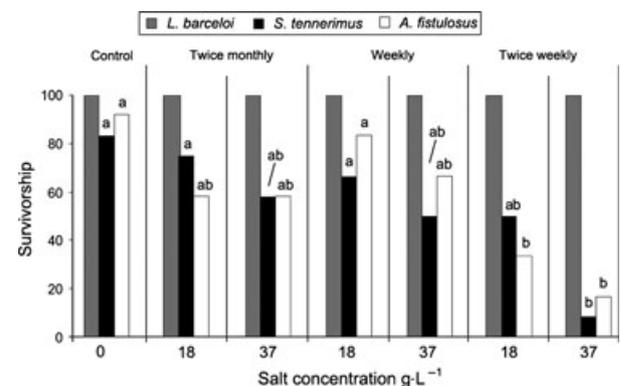


Fig. 4. Proportion of surviving seedlings of *L. barceloi*, *S. tenerrimus* and *A. fistulosus* after 28 d under six salt application regimes, differing in application frequency (twice a month, weekly, twice a week) and salt concentration per application (18 and 37 g·L⁻¹) and a control treatment (0 g·L⁻¹). Different letters indicate significant differences among salinity levels ($P \leq 0.05$).

Discussion

Our study demonstrates that invasion of ruderal plant species into salt marshes could be the consequence of enhanced germination and growth under altered salinity regimes. These opportunistic species constitute a threat to endangered salt marsh species such as *L. barceloi*. In salt marshes where salinity has decreased during critical life-history periods for salt marsh species (e.g. germination, growing season), conditions may allow the establishment and growth of taller, non-halophytic species.

Limonium barceloi is as a halophytic species with high salinity tolerance during the seedling stage, but less during germination. Previous studies of the salt tolerance of congeneric species have shown similar results, with some *Limonium* species tolerating salinity levels exceeding that of seawater (Boorman 1968; Woodell 1985; Hegazy 1992; Carter et al. 2005; Redondo-Gómez et al. 2008; Yasseen & Abu-Al-Basal 2008; Yildiz et al. 2008; Zia & Khan 2008; Lidón et al. 2009). Mean time to germination increased for *Limonium* as the salt concentration increased in this and other studies (e.g. Ungar 1978; 1982; Brenchley & Probert 1998; Gorai & Neffati 2007; Redondo-Gómez et al. 2008).

Germination, seedling performance and survival in response to different salinity levels varied by species. Interestingly, only three species were able to germinate at the highest salinity, including *L. barceloi*. Moreover, *L. barceloi* was the species with the highest capacity to germinate after a 28-day pre-treatment with seawater, which suggests that this species can survive dynamic hydrological regimes with variable substrate salinity, which are typical of salt marsh environments. However, it remains unclear whether seawater pre-treatment killed those seeds that did not germinate or whether they were still viable and may have germinated after a longer period than that examined in our study.

Although more work is needed in field conditions to study the effect of inter- and intra-specific competition for resources, it seems likely that rosettes of *L. barceloi* suffer from shading by the strong vertical growth of ruderal species examined in this study. Competition for light resources is an important limiting factor in germination of other *Limonium* species (Zia & Khan 2008). At reduced substrate salinity, *A. fistulosus* and *S. tenerrimus* may be able to invade from the edges of the environment. In contrast to the other ruderal species, seeds of these species also germinated at high salinity levels, while also tolerating low salinity as seedlings. Indeed, some individuals of these two species have been observed in the habitat once colonized by *L. barceloi* that was inundated with freshwater in 2009 (Khan & Traveset 2009a). We also suggest that *A. barbata*, *R. picroides* and *S. asper* may be able to invade, albeit these

species also have a limited ability to germinate in relatively high salt concentrations.

Experimental caveats

The findings of this study suggest that salt applications could improve the environment for endangered species such as *L. barceloi*. Nevertheless, experimental salt additions might be done off-site as a first step before applying any treatments *in situ* on remnant populations. Despite the obvious advantages of controlled experiments under standardized conditions in the laboratory and greenhouse, it is clear, however, that caution must be exercised when applying results of such studies in the field (Davy & Costa 1992). In a more complex field situation, the outcome may not be the same as in an experimental setting.

The response of species to salinity depends on life-history stage (Tobe et al. 2001). While some species may be sensitive to salinity in early life-history stages, adult individuals may tolerate hypersalinity (Beare & Zedler 1987; Callaway & Zedler 1997). Thus, adult individuals of invaded species may require hand removal. Salinity tolerance in adult plants was outside the scope of this study, but merits additional laboratory and field studies.

Conclusions and implications for management

Anthropogenic freshwater inflows into salt marshes resulting in the invasion of non-native, less salt-tolerant species threatening native halophytic species is increasingly recognized as an important conservation issue (Callaway & Zedler 1997; Greer & Stow 2003). However, few studies have tested salt application as a method to control invaders (e.g. Kuhn & Zedler 1997). Our results suggest that salt application could be an effective conservation measure to control invasive, non-halophyte species without significant negative effects on *L. barceloi*. To confirm the effectiveness of the treatments under field conditions, *ex-situ* trials should be conducted with planted *L. barcoi* and applications of 100% Mediterranean seawater administered once a fortnight. Our study suggests that life-history stages, such as germination and early seedling growth stage, may vary in sensitivity to salinity, which should be considered in future evaluations of salt application regimes. Therefore, to further minimize potential negative effects of salt application on the germination success of *L. barceloi*, we recommend the application of the saltwater treatments after germination. Based on our findings, we propose that specifically tailored salt application regimes should be more widely tested and considered as a practical conservation measure to protect rare and endangered salt marsh specialists threatened by opportunistic invaders following freshwater intrusions.

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