

Helophyte germination in a Mediterranean salt marsh: Gut-passage by ducks changes seed response to salinity

Espinar, J.L.^{1*}; García, L.V.¹; Figuerola, J.²; Green, A.J.² & Clemente, L.¹

¹Departamento de Geoecología, IRNAS-CSIC, P.O. Box 1052, E-41080 Sevilla, Spain;

²Department of Applied Biology, Estación Biológica de Doñana, Avda. de María Luisa s/n, E-41013 Sevilla, Spain;

*Corresponding author; Fax +34954624002; E-mail jlespinar@irnase.csic.es

Abstract.

Question: In seeds which are regularly consumed by waterbirds in the field, how does gut–passage modify their response to salinity gradients?

Location: Doñana National Park salt marsh, south-west of Spain.

Methods: Seeds of *Scirpus litoralis* and *Scirpus maritimus* were collected and force fed to mallards (*Anas platyrhynchos*). Both the ingested seeds (passage) and non-ingested seeds (controls) were exposed, in germination chambers, to a salinity range similar to that observed in the field (0–32 dS/m). After 30 days, the total percentage germination, the duration of the dormancy period and the germination speed were computed. The response of the different germination parameters to ingestion and salinity was analyzed using generalized lineal models. Recovery tests on seeds that did not germinate in the various treatments and tests of the effect of ingestion on the intrinsic variability in seed response were also performed.

Results: An increase in salinity reduced germinability and increased the length of dormancy, while gut passage increased the intrinsic variability of the temporal seed response in both species. In *S. litoralis* there was a significant interaction between the effects of salinity and passage on germination rate. Passage increased germination rate at low salinities (≤ 2 dS/m) but decreased it at high salinities (≥ 4 dS/m).

Conclusion: Gut-passage by ducks significantly changes seed response to salinity. The outcome of plant-animal interactions can be influenced by environmental gradients. Studies of germination in response to gut passage that do not take such gradients into account may produce misleading results.

Keywords: Doñana; Germination plasticity; Plant-animal interaction; *Scirpus*; Stress.

Abbreviation: GLM = Generalized linear model.

Introduction

Although plant-animal interactions have been a major topic in ecological research, little attention has so far been given to the indirect effects of these interactions, particularly how biotic interactions can modify the way that plant species respond to the abiotic environment (see e.g. Herrera & Pellmyr 2002).

The ingestion, and resulting dispersal, of seeds by animals is one of the most widely documented plant-animal interactions (see review by Herrera & Pellmyr 2002). The passage of seeds through the digestive tract of birds has a wide variety of effects on germination patterns (percentage germination and germination rate) among the range of plant species studied (Traveset 1998; Traveset & Verdú 2002). In most cases, germination patterns of ingested seeds differ from those of non ingested seeds, responses that are often assumed to be adaptive even though direct evidence for this assertion is rarely available (see review in Traveset 1998; Figuerola & Green in press).

Agami & Waisel (1986) and Santamaría et al. (2002) have shown that the passage of seeds of aquatic plants through the avian gut produces an increase in germination rate similar to that observed in seeds scarified mechanically. Changes in the nature of the testa due to gut-passage have been highlighted by some authors (review in Traveset 1998; see Traveset et al. 2001) and may be decisive in saline environments (e.g. Mediterranean wetlands), in which the response of the seed to osmotic stress, or its resistance to toxicity caused by some ions may be affected (Ungar 1978; Baskin & Baskin 1998).

Thus, in saline habitats, any effect of ingestion on the capacity of seeds to survive, germinate and grow is likely to vary with salinity, and a change in salinity will, in turn, modify the quality of dispersal provided by birds and the seed shadows related to bird mediated dispersal. However, we are unaware of any previous study that considers how the effects of ingestion on seeds may be influenced by salinity.

In this paper we analyse the effects of ingestion by ducks on the patterns of seed germination of two helophyte species and how birds can modify the response of plants in the face of environmental stress, represented by a gradient of salinity similar to those found in natural habitats.

Methods

Study site and species

The plant species studied (*Scirpus maritimus* and *S. littoralis*) are the major components of the perennial vegetation in the temporary marshes in Doñana National Park, a non-tidal coastal marsh with a Mediterranean climate situated in southwestern Spain (see Espinar et al. 2002 and García et al. 1993). In both species, the shoot grows up through the water column from a rhizome once the wet season begins (autumn), fruits (in late spring) then loses its aerial parts during the course of the dry season (summer).

Water birds are very abundant in the study area, with annual winter counts of migratory *Anatidae* alone exceeding 300 000 individuals from a variety of species (Martí & del Moral 2002), many of which consume important quantities of seeds of *Scirpus* spp. (Green et al. 2002). One of the most abundant species is the duck *Anas platyrhynchos* for which *Scirpus* spp. seeds are an large component of the diet (Green & Selva 2000; Green et al. 2002). In Doñana, intact *Scirpus* seeds are often found in waterfowl faeces (Figuerola et al. 2003) and 19% of 186 gizzards of ducks and coots from Doñana contained *Scirpus* spp. seeds (J. Figuerola et al. unpubl. data).

Seed collection and pre-treatment

In August 2001, ten populations of each helophyte species were selected at random from an area of 6000 ha in the southern part of the marshes of Doñana National Park. From each population, the inflorescences of 60 shoots were collected at random, with each shoot separated from each other by at least 10 m. In the laboratory, the seeds were separated from the spikes and those coming from distinct populations were mixed to a homogeneous sample representative of the study area. The seeds were stored in darkness at 4 °C in order to stimulate germination (Clevering 1995).

Seeds were given to 20 semi-captive ducks. On 24 March 2002 the birds were distributed in individual cages with a mesh floor and given unlimited access to food (broken rice) and drinking water. Removable

metal trays were placed under the cages to recover any droppings. The next morning, ca. 500 *S. littoralis* seeds were force fed to the birds. The following day, faeces were collected from the trays and transported in plastic bags to the laboratory. The mallards were kept in a large pen until 9 April 2002, when the process was repeated with the same individuals. In this case each bird was fed with 500 *S. maritimus* seeds. After the collection of faeces the birds were returned to large ponds in the wildlife centre.

The faeces collected were washed in a sieve then dried at room temperature. Intact seeds were separated and stored in darkness at 4 °C.

Germination experiments

Saline solutions used in the experiment were prepared by adding different quantities of sea salt (dried to 110 °C for 24 hr) to demineralized water to produce the range of salinities observed in the field (< 1 to 30 deciSiemens per meter (dS/m)). Seven different saline solutions were used with electrical conductivities (EC) of 0, 1, 2, 4, 8, 16 and 32 dS/m.

Both the ingested (passage) and non-ingested (controls) seeds were separated at random into groups of 25 seeds, using four groups for each of the seven salinity treatments employed (four replicates with a total of 100 seeds for each combination of ingestion treatment and salinity, i.e. 700 ingested seeds and 700 controls for each plant species). Ingested seeds were subsampled from those ingested by different ducks so that each of the 20 individuals contributed 35 ingested seeds per plant species to the germination trials. Individual ducks vary greatly in the proportion of seeds that are destroyed during digestion (see Figuerola et al. 2002; Santamaría et al. 2002) and this stratification was used to remove the possibility that individual ducks destroying fewer seeds during ingestion were contributing relatively more to the sample of seeds used in our experiments.

Each group of seeds was placed in a Petri dish containing a filter paper and 20 ml demineralized water or saline solution. In the case of *S. littoralis*, a significant proportion of seeds germinate only if they are submerged (Espinar et al. unpubl. data), and thus 250 ml precipitation flasks were used to keep the seeds continuously submerged.

The total of 112 dishes and flasks containing the seeds were placed in a germination chamber, with a 12 hr/12 hr light-darkness photoperiod and a light intensity of 200 $\mu\text{mol}\cdot\text{m}^{-2}$ photons and temperature cycles of 25 °C/10 °C. Every two days the number of seeds germinated on each dish/flask was observed to obtain, after 30 days, the total percentage germination (G_T). For each treatment we calculated the number of days taken for the

first seed to germinate (T_0) as an estimate of dormancy, and the time (in days) taken for half of those seeds that germinated by the end of the experiment to germinate (T_{50}), as an estimate of the speed of germination (Barnea et al. 1991; Traveset et al. 2001).

To detect a possible loss of viability of ingested seeds that did not germinate in the various treatments, they were placed in a freshwater medium for a month at 4 °C and in darkness, they were then placed in a non-saline medium to germinate using the above protocols (recovery test), calculating the total percentage germination after 30 days (G_R). The seeds used for these were those which did not germinate in the first germination trial under variable salinity concentrations.

Data analysis

The response of the different germination parameters to passage and salinity was modelled using generalized lineal models (GLM). Given the nature of response variables (counts) a Poisson distribution with log-link was assumed (Dobson 2002). For each response variable the model included two fixed independent variables and the interaction between them. The bird treatment was modelled as a categorical variable with two categories (ingested and control seeds) and salinity as a continuous variable. As a preliminary guide for modelling salinity effects, local polynomials were fitted (DWLS; McLain 1974) and projected on salinity-germination response scatters. Then, appropriate polynomial (lineal, quadratic or cubic) effects of salinity, together with passage and first order interaction (salinity \times passage) effects on germination parameters were tested using GLM (Wald test, see Dobson 2002). The sign of parameters having significant effects was used to interpret the results.

The fit of the overall model for the estimated Poisson

regression model was expressed with the conventional R -statistic. For each model, the Pearson r -coefficient value between the observed and predicted response was computed. When the assumptions for a parametric approach were not fulfilled by the data, the significance of the r -values obtained was tested by re-sampling (Simon 1999). The effect of *passage* on the intrinsic variability in the response of seeds of each species to the experimental salinity gradient was tested using a non-parametric approximation (bootstrap, Mooney & Duval 1993) to Lewontin's (1966) test. The effect of the recovery period on the ingested seeds of each species was analysed with a Wilcoxon test.

To avoid the inflation of 'tablewise' type I error rate, we applied sequential-Bonferroni corrections (Holm 1979) when repeated tests were carried out.

Results

Effects of salinity and gut-passage on seed germination

Overall, salinity and gut-passage explain a significant fraction of the observed response in the different germination parameters ($p < 0.0005$ in all cases, Table 1).

Percentage germination (G_T)

In both studied species there was a decline in germinability (G_T) at salinities above 1-2 dS/m (Table 1, Figs. 1 and 2).

In *S. maritimus* (Fig. 1) ingestion did not have a significant effect on germinability or on the way that seeds responded to increasing salinity (Table 1). *S. litoralis* (Fig. 2) also showed no direct effect of *passage* on the total germination percentage, but *passage* affected the response of seeds to salinity (Table 1).

Table 1. Log-linear regression models of the values of germination parameters (G_T, T_0, T_{50}) in two *Scirpus* species, as a function of the salinity in the medium (S), passage (P) and the interaction between both factors (SP). Only parameters with significant ($p < 0.05$, Wald test) partial effects are included. The direction (sign) and p -values are shown in bold for effects which remained significant after Bonferroni sequential correction (Holm 1979).

| | <i>Scirpus maritimus</i> | | | | | | <i>Scirpus litoralis</i> | | | | | |
|----------|--------------------------|--------------|--------|------|--------------|------|--------------------------|--------------|--------|--------------|-------|------|
| | Whole model | | Factor | | | | Whole model | | Factor | | | |
| | R | p | Effect | Wald | p | Sign | R | p | Effect | Wald | p | Sign |
| G_T | 0.90 | 0.000 | S^2 | 13.5 | 0.000 | - | 0.82 | 0.000 | S^2 | 38.4 | 0.000 | - |
| | | | | | | | | P | 0.5 | 0.003 | - | |
| T_0 | 0.80 | 0.000 | S | 44.1 | 0.000 | + | 0.85 | 0.000 | S^2 | 7.0 | 0.008 | - |
| | | | P | 8.9 | 0.003 | - | | S | 11.5 | 0.001 | + | |
| | | | SP | 4.7 | 0.030 | + | | P | 14.0 | 0.000 | - | |
| T_{50} | 0.67 | 0.000 | S | 8.7 | 0.003 | + | 0.77 | 0.000 | S^2 | 4.4 | 0.035 | - |
| | | | | | | | | S | 7.6 | 0.006 | + | |
| | | | | | | | | P | 6.6 | 0.011 | - | |
| | | | | | | | | SP | 11.1 | 0.001 | + | |

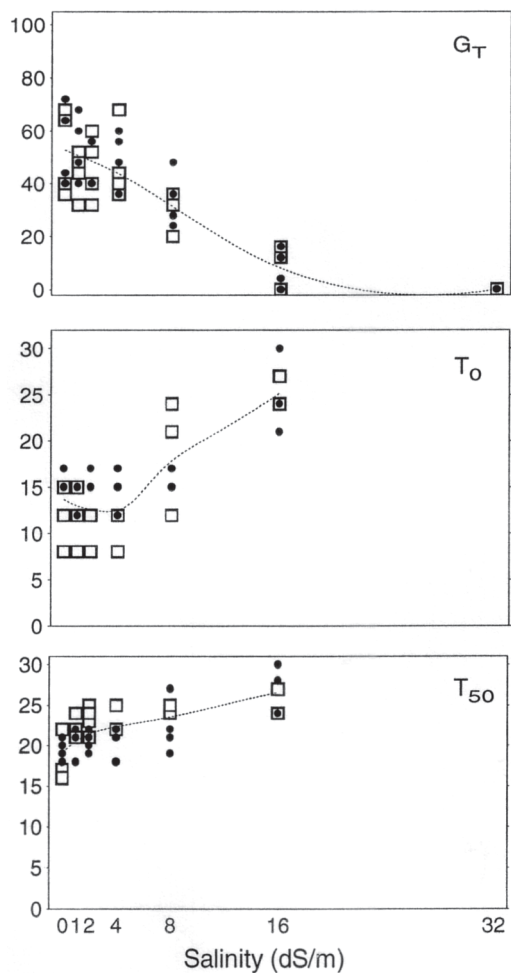


Fig. 1. Values of germinability (G_T , %) and number of days until the first seed germinated (T_0) and 50% of total germination (T_{50}) was reached, for ingested (\square) and control (\bullet) seeds of *Scirpus maritimus*. Overall distance weighted least squares regression lines are shown.

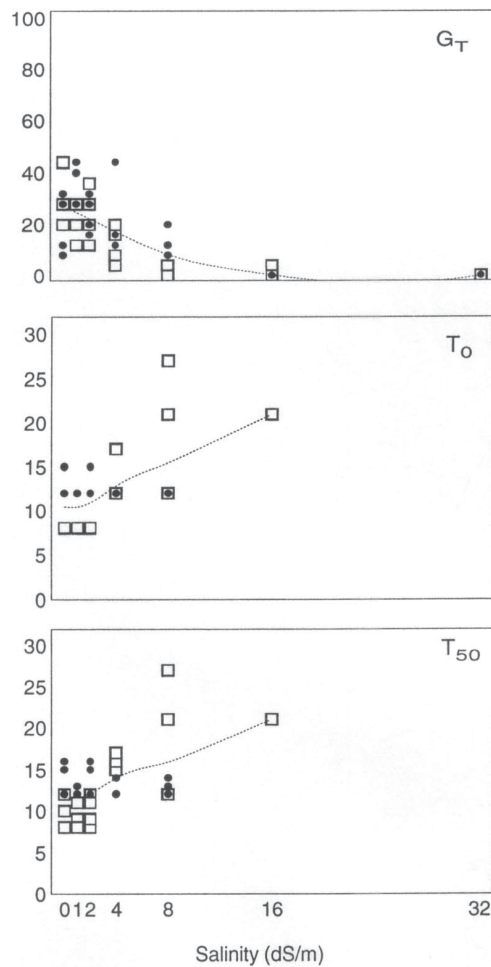


Fig. 2. Values of germinability (G_T , %) and number of days until the first seed germinated (T_0) and 50% of total germination (T_{50}) was reached for ingested (\square) and control (\bullet) seeds of *Scirpus litoralis*. Overall distance weighted least squares regression lines are shown.

Table 2. Results of the recovery test for ingested seeds, showing the salinity (dS/m) used for the initial treatment (salinity), percentage of seeds that recovered after transfer to non-saline conditions (N_R) and cumulative percentage of seeds that germinated after both periods (G_{FINAL}).

| Salinity | <i>Scirpus maritimus</i> | | <i>Scirpus litoralis</i> | |
|----------|--------------------------|-------------|--------------------------|-------------|
| | N_R | G_{final} | N_R | G_{final} |
| 0 | 2 | 53 | 31 | 67 |
| 1 | 39 | 66 | 39 | 66 |
| 2 | 22 | 58 | 24 | 59 |
| 4 | 47 | 72 | 58 | 78 |
| 8 | 27 | 47 | 59 | 70 |
| 16 | 56 | 59 | 29 | 34 |
| 32 | 55 | 55 | 50 | 50 |
| Mean | 36 | 59 | 42 | 61 |

Table 3. Overall response (mean and coefficient of variation, CV) of *Scirpus* species to gut-passage treatment. Differences that were significant after applying the Bonferroni sequential correction are in bold.

| | <i>Scirpus maritimus</i> | | | <i>Scirpus litoralis</i> | | |
|---------|--------------------------|--------------|----------|--------------------------|--------------|--------------|
| | G_T | T_0 | T_{50} | G_T | T_0 | T_{50} |
| Mean | | | | | | |
| Control | 36 | 17 | 21 | 17 | 12 | 13 |
| Passage | 32 | 14 | 23 | 13 | 12 | 13 |
| p | 0.429 | 0.005 | 0.039 | 0.335 | 0.029 | 0.243 |
| CV | | | | | | |
| Control | 1.01 | 0.23 | 0.15 | 0.86 | 0.09 | 0.11 |
| Passage | 0.69 | 0.45 | 0.13 | 1.01 | 0.49 | 0.40 |
| p | 0.823 | 0.001 | 0.512 | 0.029 | 0.000 | 0.000 |

In both species, a large proportion of defecated seeds which did not germinate in the most saline media underwent germination after being transferred to a more favourable medium, the accumulated percentage germination being similar to that obtained for seeds that were placed in low salinity media from the start. In both species, the total number of seeds that germinated was significantly higher following the recovery period ($p < 0.018$ for both). The lack of a response of ingested seeds to the most saline treatments does not seem to be related to a permanent loss of viability but to a transient inhibition of germination until more favourable conditions appear (Table 2).

Rate of germination (T_0 and T_{50})

In general, an increase in salinity tended to delay the start of germination (T_0), while *passage* tended to accelerate it (Table 1, Figs. 1 and 2).

In *S. maritimus*, salinity significantly delays the start of germination, while ingestion produces an independent effect in the opposite direction (Table 1). In *S. littoralis*, both the main effects and the interaction between them have a significant effect on T_0 (Table 1).

In both species (Figs. 1 and 2) ingestion does not affect germination speed, although this is reduced by an increase in salinity (Table 1). Unlike *S. maritimus*, in *S. littoralis* the response to salinity was indirectly modified by the effect of *passage*. In low salinity media (< 2 dS/m) the germination rate of ingested seeds increased compared to that of control seeds, but when the salinity was higher (≥ 4 dS/m) the germination rate decreased in a marked way (Fig. 3).

Variability in the germination response

Table 3 shows the overall effect of *passage* on the mean value and relative variability of the response of seeds of the two studied species across the whole salinity gradient combined.

In *S. littoralis*, passage increased the relative variability in T_0 by over five times and T_{50} by almost four times, but did not affect the mean value of either parameter across the whole salinity gradient. In *S. maritimus*, passage doubled the relative variability in T_0 and reduced its mean value significantly.

Discussion

The effect of salinity and gut passage on germination parameters

In the helophyte species studied, an increase in salinity produces a decrease in the percentage of seeds that germinate and an increase in the duration of seed dormancy, as observed in studies of other species from saline habitats (Ungar 1978; Marañón et al. 1989). The only direct effect of passage is the reduction in the duration of seed dormancy. Izhaki & Safriel (1990), Traveset et al. (2001) and Santamaría et al. (2002) have obtained similar results in seeds of other Mediterranean plant species frequently consumed by birds.

The passage of seeds through the digestive tract of *A. platyrhynchos* directly affects the mechanisms that control the dormancy period in the two *Scirpus* species, causing a reduction in the reaction time of seeds under conditions favourable for germination, but without affecting the total proportion of seeds that germinate.

This suggests that, once the price of transport caused by the loss of propagules digested by birds has been

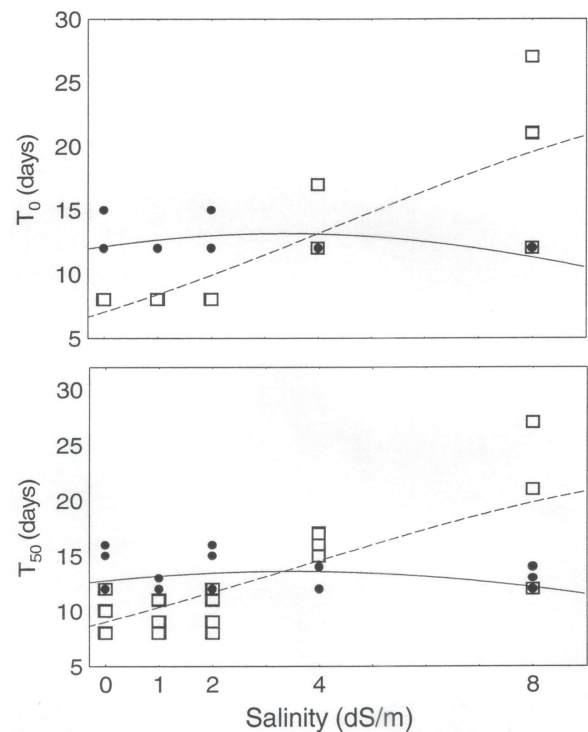


Fig. 3. Observed and modelled response to salinity of the ingested (\square) and control (\bullet) seeds of *Scirpus littoralis*. Upper graph: salinity against dormancy period length (T_0). Lower graph: salinity against an inverse measure of germination speed (T_{50}).

paid, these *Scirpus* species do not suffer an additional cost from a loss of viability of transported seeds. The seeds of these two species are, amongst those of helophytes, the most frequently consumed by waterfowl in marshes of the Mediterranean region (Suarez & Urios 1999; Green et al. 2002; Fuentes et al. in press).

Some authors have suggested that, in ecosystems in which the major environmental variables change in an unpredictable way, the increase in variability of the response of seeds itself favours the survival of plants (Izhaki & Safriel 1990; Traveset et al. 2001; Santamaría et al. 2002). This increase in the variability of the response can be especially favourable when the seeds are transported to areas away from the mother population, in which the range of environmental variables can differ from that in the original habitat. Locally, close to or within the mother population, most of the seedbank will constitute non-ingested seeds and only a small fraction can be expected to consist of seeds that have undergone passage. The presence of such ingested seeds will increase the polymorphism in germination parameters that usually show little variation, without having a major effect on the overall response of the seedbank (i.e. without affecting the mean response acquired by selective pressure).

Gut-passage and salinity-modulated response in Scirpus litoralis

Our results show that the response of *S. litoralis* seeds to the factors studied is the most complicated, with ingestion having major indirect effects on the regulation of dormancy period and germination rate, in contrast to the direct effects that predominate in *S. maritimus*.

Apparently, passage increases the sensitivity of *S. litoralis* seeds to salinity, resulting in a decrease in the percentage germination above 4 dS/m. Nevertheless, the results of the recovery test show that the reduction of germinability in highly saline media is reversible (at least up to 32 dS/m) and that the seeds retain their capacity to germinate if the salinity in the environment is reduced at a later date.

The only direct effect of passage in *S. litoralis* seeds (reduction in the dormancy period T_0) is itself subjected to an interaction with the salinity of the environment (Table 1). Thus, compared to controls, the ingested seeds have a reduced T_0 when the salinity is low (≤ 2 dS/m) and extend it when the salinity exceeds 4 dS/m.

The changes induced by passage to the rate of germination (T_{50}) in *S. litoralis* also depend on salinity. A more saline environment reduces the rate for ingested seeds (i.e. increases T_{50}) compared to controls, whilst a less saline one increase it (i.e. decreases T_{50}).

If the changes in the temporal response of seeds is compared along the salinity gradient (Fig. 3), it is clear that the response (as measured by the magnitude of T_0 and T_{50}) of ingested seeds can be lower (in more favourable conditions), higher (in the most saline environments) or the same (intermediate values) as that of control seeds. It is noteworthy that the range of salinities in which the response of ingested and control seeds is similar (3-4 dS/m) approaches the mean salinity recorded in spring in the established populations from which seeds were obtained (Fig. 3).

An advantageous interaction?

The dispersal of seeds by birds can produce clear benefits for the plants e.g. by allowing the colonization of new areas, avoidance of sibling and mother-sibling competition and permitting gene flow between isolated or highly fragmented populations (Howe & Smallwood 1982). It also has costs due to loss of propagules or their displacement to habitats unsuitable for germination (Mueller & van der Valk 2002; Santamaría 2002; Figuerola et al. 2002). However, to show that modifications caused by passage to key reproductive parameters of plants (i.e. germination parameters) may be adaptive, there is a need to show that these changes promote the establishment and survival of seedlings (Schupp 1993). This is very difficult to do given the high spatial and temporal variability in the habitats frequented by the birds, the number of environmental variables involved and the diversity of strategies found in different plants. Consequently, as yet we cannot generalise from our results as to whether the final outcome of the plant-animal interactions is positive or negative for the plant.

Our results support our two initial working hypotheses: that passage alters the response of seeds, and causes important changes in their response to one of the environmental variables that limit sexual reproduction in Mediterranean marshes.

Salinity is a key determinant of the organization of plant communities in Mediterranean coastal marshes (Callaway et al. 1990; Grillas 1990; García et al. 1993), which are subjected to strong oscillations within and between years, associated with seasonal flooding-drought cycles. The influence of salinity is decisive during the reproductive period, both for the germination of seeds (Ungar 1998) and the establishment of seedlings (Bertness & Yeh 1994; Noe & Zedler 2001). A greater plasticity in the response of the seeds in the face of a key environmental parameter (which strongly conditions the life of plants in these habitats) may potentially promote the establishment of seedlings, especially when seeds are moved to areas different from those occupied by the mother population.

Furthermore, in the case of *S. litoralis*, passage modifies the temporal response of seeds to a salinity gradient. Instead of having their response modified in a uniform way, ingested seeds may or may not respond in a similar way to non-ingested seeds, depending on the salinity.

The possible advantages and disadvantages associated with changes in the dormancy period and germination rate of the seeds ingested by birds has been discussed by several authors for specific species and environmental variables (review in Traveset 1998; Figuerola & Green in press). Our results show that the possible effects of bird-seed interactions should be studied from a dynamic perspective. To shed light on the real importance of these interactions in terms of germination patterns and individual fitness, it is essential to analyse the response of the seeds (and seedlings) in the context of fluctuations in environmental variables observed in the field. The relevance of many previous laboratory studies on the effects of gut-passage on aquatic plants that do not address possible interactions with salinity or other environmental factors is open to discussion.

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