# Effects of seed ingestion and herbivory by waterfowl on seedling establishment: a field experiment with wigeongrass *Ruppia maritima* in Doñana, south-west Spain

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## Abstract

The ingestion of seeds by vertebrates usually affects the viability and/or germination rate of seeds. Increases in germination rate following passage through the vertebrate gut have often been assumed to be favourable for seedling survival and plant fitness, but this assumption has never been tested experimentally. Given that numbers of herbivorous waterfowl are higher in winter in Mediterranean wetlands, herbivory pressure there will be higher for early growing plants. In a factorial experiment we investigated the effects of seed ingestion by ducks (shoveler, *Anas clypeata*) on the survival of wigeongrass *Ruppia maritima* seedlings in the field in Doñana (south-west Spain), under differing exposures to herbivory by waterfowl and fish. We planted ingested and non-ingested seeds in December, using exclosures to protect half of them from herbivores. When they were protected inside exclosures, there was no difference between ingested and non-ingested seeds in the number of plants that survived until June-July. However, fewer plants survived from ingested seeds when exposed to natural levels of herbivory because they were exposed for longer than plants germinating from non-ingested seeds. In conclusion, increases in germination rate after ingestion are not necessarily beneficial for the plant, and the final outcome depends on complex interactions with other factors such as herbivore abundance.

## Introduction

The dispersal of seeds is an important ecological process and the effects of ingestion by vertebrates on the capacity of seeds to germinate have been studied in many systems. Ingestion by vertebrates usually affects the germinability of seeds (i.e., proportion of seeds that germinate), or the rate of germination (i.e., inverse of the time between seeding and start of germination, see review by Traveset (1998)). Traditionally, faster germination after ingestion has been considered as intrinsically beneficial for the plant (but see discussion in Traveset (1998)). This assumption is partly supported by the high growth rate and higher survival of seedlings germinating earlier (Zimmerman and Weis 1984; Waller 1985; Bush and Van

Auken 1991; Seiwa 1998). However, these relationships between early germination and fitness have been deduced from natural variation in germination times of non-ingested seeds. Early germination may also result in different risks due to adverse climatic conditions or a higher probability of predation or pathogen attack (Jones and Sharitz 1989; Traveset 1990). However, to the best of our knowledge, no field data are available on the success of ingested seeds in comparison to non ingested seeds in any system.

The final effects of germination phenology on fitness are likely to depend on the characteristics of each species and the conditions available for establishment in a given area. Field experiments are necessary to determine the ultimate consequences of seed inges-

tion by vertebrates in terms of seedling survival or reproductive success. The influence of herbivory is one variable that needs to be addressed in such experiments. Herbivore pressure can affect the interactions between plants and their pollinators (Herrera 2000), change the optimal flowering phenology (Pilson 2000), and determine the structure and diversity of plant communities (Huntly 1991). In the case of submerged macrophytes, the advancement of germination time may expose seedlings to higher densities of migratory herbivorous waterfowl (Lodge et al. 1992; Idestam-Almquist 1998). In a recent review, Marklund et al. (2002) concluded that the impact of waterfowl on submerged macrophytes is most important during the colonisation phase and at high bird densities.

Wigeongrass *Ruppia maritima* (L.) is an aquatic angiosperm that inhabits brackish coastal and inland saline waters with a sub-cosmopolitan distribution (Verhoeven 1979). Mechanisms of dispersal of *Ruppia* spp. are not well understood, but their seeds and green parts are important waterfowl foods (Gaevskaya 1966; Cramp and Simmons 1977). Agami and Waisel (1988) demonstrated that seeds can resist passage through the gut of fish. In the same Mediterranean wetland used in the current study, Figuerola et al. (2002) reported the presence of undamaged seeds of *Ruppia maritima* in 23% and 36% of the waterfowl droppings examined in early (November-December) and late (February) winter respectively. The rate at which seeds of *Ruppia maritima* germinated over time increased for duck ingested seeds as compared to control (non-ingested) seeds (Figuerola et al. 2002). The proportion of seeds germinating after ingestion by shoveler *Anas clypeata* was the same as for control seeds (Figuerola et al. 2002).

In this paper we study the consequences of *Ruppia maritima* seed ingestion by ducks in a field experiment while manipulating herbivore pressure. We test whether ingestion by ducks enhances the establishment of seedlings, and whether herbivory reduces (or reverses) any benefits of ingestion by decreasing seedling survival.

## Methods

The study was performed in 'Veta la Palma' (36°57′ N, 6°14′ W), a brackish marsh in Doñana, south-west Spain. The area includes 37 rectangular ponds (total surface area 3,125 ha) managed for fish farming, and
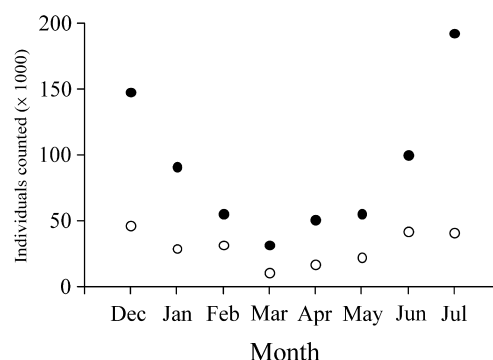


*Figure 1.* Results of waterfowl counts in Veta la Palma from December 1999 to August 2000. Filled circles show the total number of waterfowl (*sensu* Rose and Scott (1997): ducks, coots, waders, gulls, herons, flamingos, spoonbills, *etc*) and open circles to the number of ducks and coot. Source: Equipo de Seguimiento de Procesos Naturales, Estación Biológica de Doñana.

4,442 ha of untransformed temporary marshes. Salinity ranged from 10 to 17 g/l at the time of study. The ponds contain estuarine fish such as *Dicentrarchus labrax*, *Mugil cephalus* and *Sparus auratus* at unknown densities. The area is very important for waterfowl (*sensu* Rose and Scott (1997): ducks, coots, waders, gulls, herons, flamingos, spoonbills, *etc*) and most of the waterfowl in Doñana often concentrate in these ponds due to the scarcity of natural, seasonal habitats. Waterfowl were counted monthly in Veta la Palma during our study via ground surveys (Figure 1).

In September 1999, *Ruppia maritima* seeds were collected from the shores of several ponds. The seeds were separated from debris and stored dry in plastic vials in the refrigerator. In December 1999, seeds were force-fed to three captive shovelers (*Anas clypeata*) and the droppings were collected the next day. Immediately after collection, droppings were sieved (mesh-size: 0.5 mm) and groups of 10 whole seeds were extracted and stored dry in separate tubes in the refrigerator until planting in the field. Three such feeding sessions were conducted on successive days to obtain 240 duck ingested seeds.

The effects of gut passage and herbivory on plant establishment were studied simultaneously with a factorial experiment in two ponds in the field. Plastic pots were filled up to five cm below the rim with sediment from the study area which had been passed through an autoclave for 30 minutes to kill any propagules present. On 12 December 1999, ten *Ruppia* seeds were planted in each of 48 pots, pushing them down to a depth of one cm below the sediment

surface. The pots were then partially buried at the bottom of the ponds, leaving five cm rising above the surface of the mud to protect the contents from water currents. In each pond we used six blocks and in each block we used four pots. The pots within a block were distributed on the four corners of an imaginary square of 50 cm × 50 cm. Duck ingested seeds were planted in two pots selected at random, and non-ingested control seeds were planted in the other two. Within each block, one ″control″ and one ″ingested″ pot were placed inside a wire mesh cage (1 × 0.5 m, mesh size 1 cm) as the ″no herbivory″ treatment. These exclosures were high enough to reach the water surface. The ″herbivory″ treatment consisted of the two pots outside the exclosure. The position of treatments within each block was changed at random.

Thus, overall we used four experimental treatments: 1) control seeds exposed to herbivory, 2) duck-ingested seeds exposed to herbivory, 3) control seeds protected from herbivory and 4) ingested seeds protected from herbivory. Although we excluded herbivorous birds and large fish in treatments 3 and 4, plants in all treatments were potentially exposed to herbivory by small fish or invertebrates.

The resulting plant samples from the first pond were collected on 3 June 2000 and from the second pond on 17 July. The plants were well grown and had reached the water surface, but had not completed seed production (which spans from June to September in La Camargue at 46° N, Verhoeven (1979)). The number of plants in each pot was counted. Germination could not be monitored during the experiment because high water turbidity made it impossible to check the pots without disturbing the experiment. Our intention was to measure biomass of each sample, but most of the samples were lost during a fire in the laboratory.

*Statistical analyses*

The significance of the effects of herbivory and duck-ingestion on the number of plants in each pot were assessed by applying a General Linear Model with repeated measures. Since the count data were highly skewed due to the abundance of zeros (pots with no plants growing), a negative binomial error structure and a log link function were used in the model (Crawley 1993). A repeated SUBJECT effect was included in the model to clump the data from the four treatments within each independent block (see Stokes et al. (1995) and SAS Institute Inc (1996)). Blocks were

nested within a pond effect to control for the different collection dates and conditions in the two study ponds. Main effects (duck ingestion and herbivory treatments) and the two-way interaction were fitted using type III sum of squares, and tested using the chi-square distribution (SAS Institute Inc 2000). All calculations were done with SAS version 8.2 (SAS Institute Inc 2000). Overall, the design used was similar to an ANOVA design with randomised blocks (see Zar (1996)), but the use of GLM methods was necessary because the count data did not fit the normality assumption of traditional ANOVA methods (Crawley 1993).

## Results

In June-July, a significant effect of duck digestion was detected on the number of plants growing in the pots, with less plants in pots with ingested seeds ($\chi^2 = 4.05$, p = 0.04). Although the herbivore exclusion treatment did not have a significant effect on the number of plants growing ($\chi^2 = 0.16$, p = 0.69), the herbivory and digestion treatments showed a significant interaction ($\chi^2 = 4.22$, p = 0.04). This interaction was due to fewer plants in the pots with duck-ingested seeds, but only when exposed to herbivory ($\chi^2 = 12.30$, p = 0.0005, see Figure 2). Control (non-ingested) and ingested seeds had the same survival rate when protected from herbivory ($\chi^2 = 0.00$, p = 1.00). The number of plants found in pots planted with control seeds was not significantly affected by the herbivore treatment ($\chi^2 = 1.42$, p = 0.23), but for duck ingested seeds there was a tendency for more plants to occur when protected from herbivory ($\chi^2 = 3.75$, p = 0.05).

## Discussion

When *Ruppia maritima* plants were exposed to natural levels of herbivory (albeit in an artificial marsh), plants growing from duck ingested seeds showed lower survival and produced fewer recruits in the population than those growing from non-ingested seeds. However, under reduced herbivory plants growing from ingested and non-ingested seeds showed equal survival. We suggest that this difference was due to the increase in germination speed of seeds of *Ruppia maritima* ingested by ducks, already demonstrated by us in the same study site (Figuerola et al. 2002). Germination of undigested *Ruppia marit-*
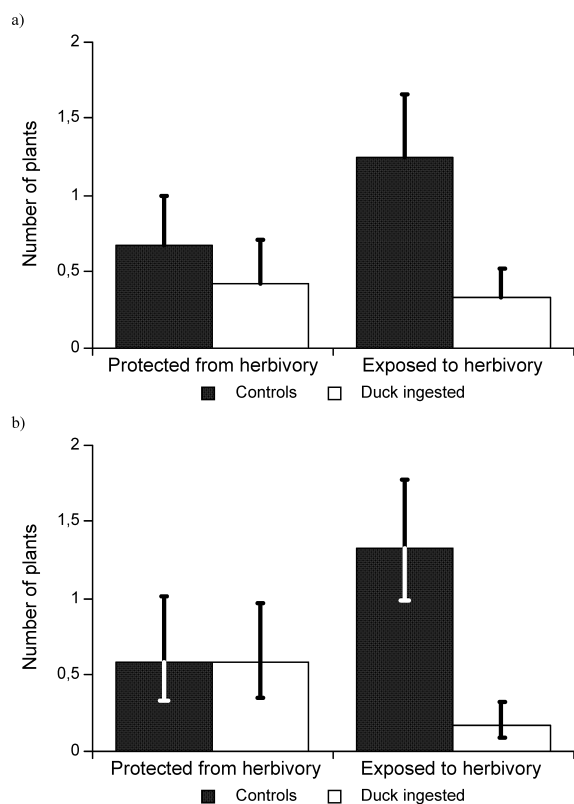
a)



b)



*Figure 2.* a) Mean number of *Ruppia* plants growing for each treatment with bars showing standard errors. Seeds ingested by *Anas clypeata* and control (non-ingested) seeds were planted inside exclosures protecting plants from herbivory, or exposed to natural levels of herbivory by waterfowl and fish. b) Mean number of plants growing for each treatment (and standard errors), after controlling for differences between blocks in a GLM with a negative binomial error distribution and a log link (see methods).

*ima* seeds occurs by mid February-early March in southern France (Verhoeven 1979), and earlier in Doñana where day length is longer and temperatures are warmer. We have found extensive germination by mid February in our study site. Under laboratory conditions at 20 °C, seeds extracted from *Anas clypeata* faeces had shorter germination time than undigested seeds (50% percentiles: 0 days for ingested seeds, 7 for control seeds; 100% percentiles: 7 for ingested seeds, 21 for controls; data from Figuerola et al. (2002), $P < 0.0001$). Unlike control seeds, many ingested seeds germinated in the refrigerator at 5 °C before the initiation of germination trials.

Thus, the earlier germination of ingested seeds exposes seedlings to herbivory for more days during the winter period of higher waterfowl densities (see Figure 1), at a time when water temperatures and *Ruppia*

growth rates are relatively low (Verhoeven 1979). Seeds germinating later in the season suffer a lower risk of predation and less time overlap between plant growth and high waterfowl densities. By the end of March, most herbivorous waterfowl wintering in or migrating through Doñana have left on their way to breeding areas in northern Europe (Figure 1, Scott and Rose (1996)). Ingested seeds protected in exclosures were not exposed to a greater risk of herbivory and showed similar survival to control seeds. This suggests that, in our study, the influence of early germination on plant establishment was unrelated to climatic effects, and shows that the differences in the survival of plants growing from ingested and non-ingested seeds were not due to differences in the number of seeds germinating for each treatment.

Herbivory commonly affects the hierarchies in plant communities (Huntly 1991), and our study suggests that this can apply at an intraspecific level by reducing the possibilities of establishment of those seeds ingested by waterfowl. Nevertheless, seed ingestion by waterfowl could provide benefits from escape (deposition of the seeds away from the mother plant), directional (transport to favourable areas for germination) and colonisation (transport of the seeds to new localities) effects of seed dispersal (Howe and Smallwood 1982; Figuerola and Green 2002). However, these benefits will be countered by costs derived from exposure to a greater risk of mortality due to herbivory.

Asynchronous germination can be favoured in environments with unpredictable climatic conditions (Harper 1977), as is the case in seasonal Mediterranean wetlands that tend to have unpredictable flooding cycles (Pearce and Crivelli 1994). Seed ingestion by vertebrates result in a diversification of germination patterns. In dry years it is possible that plants germinating from ingested seeds have a higher fitness than non ingested seeds because only early growing plants are able to complete the seed production cycle before a wetland dries out. Furthermore, spatial variation in the density of herbivores is likely to change the results of the interaction between germination rate and herbivory. In our study site, another exclosure experiment in natural *Ruppia* beds has shown that grazing by waterfowl has a strong effect on *Ruppia* biomass and survival (authors, unpublished data). However, similar experiments on coastal marshes in Louisiana found that the effects of waterfowl on *Ruppia* were undetectable (Hunter 2000). Several other studies have failed to find any influence of waterfowl

on submerged macrophytes (Marklund et al. 2002). In such low herbivory conditions, early germination might provide benefits via competition for light and space, as has been demonstrated in other species (e.g., Garwood (1983) and Jones and Sharitz (1989)). In addition, other factors such as salinity, temperature and turbidity can influence *Ruppia* growth (Verhoeven 1979; Santamaría et al. 1996; Santamaría and Hootsmans 1998), and may in turn influence the consequences of the herbivory-duck ingestion interaction.

In conclusion, increases in germination rate after seed ingestion by waterfowl are not necessarily beneficial. Among other potential sources of variation, herbivore abundance has major consequences for the influence of seed ingestion on plant fitness.

## Acknowledgements

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