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Internal transport of alien and native plants by geese and ducks: an experimental study

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SUMMARY

1. Alien plant species are rapidly spreading in aquatic ecosystems around the world, causing major ecological effects. They are typically introduced by humans, after which natural vectors facilitate their further spread. Migratory waterbirds have long been recognised as important dispersal vectors for native and aquatic plants, yet little is known about their role in the spread of alien species.
2. We determined experimentally the potential for long-distance dispersal of native and alien wetland plants in Europe by two abundant waterfowl: mallards *Anas platyrhynchos* and greylag geese *Anser anser*. We fed seeds from two plants alien to Europe and two native plants to 10 individuals of each bird species, testing for the effects of bird and plant species on the potential for dispersal.
3. Intact seeds were retrieved from faeces for up to 4 days after ingestion. The proportion of seeds retrieved intact varied significantly between plant, but not bird, species. Retrieval was highest for the invasive water primrose *Ludwigia grandiflora* (>35% of ingested seeds), lowest for the invasive cordgrass *Spartina densiflora* (<3%) and intermediate for the native glasswort *Arthrocnemum macrostachyum* and seablite *Suaeda vera* (5–10%).
4. Seed retrieval patterns over time varied between both plant and bird species. Contrary to expectations, seeds were retained in the gut for longer in the smaller mallards. No *Spartina* seeds germinated after retention for over 8 h, whereas some seeds of the other species germinated even after retention for 72 h. Germinability was reduced by gut passage for *Ludwigia* and *Arthrocnemum* seeds. *Ludwigia* seeds recovered from geese were more likely to germinate than those recovered from mallards. Time to germination was reduced by gut passage for *Spartina* and *Ludwigia*, but increased with retention time.
5. Ducks and geese evidently have the potential for long-distance transport of alien and native plant seeds, with maximal dispersal distances of well over 1000 km. The much greater potential of *Ludwigia* than *Spartina* for dispersal by waterfowl is consistent with its faster expansion across Europe. Maximum retention times of wetland seeds have been underestimated in previous experimental studies that lasted only 1–2 days. Contrary to previous studies, wetland plants with large seeds, such as *Ludwigia*, can still show high potential for long-distance dispersal. More attention should be paid to the role of waterbirds as vectors of alien plants and to the role of migratory geese as vectors of plants in general.

Keywords: endozoochory, *Ludwigia*, plant invasions, seed dispersal, *Spartina*

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Introduction

Seed dispersal is possibly the most important ecosystem service provided by birds (Sekercioglu, 2006; Green & Elmberg, 2014). In recent decades, a great deal of research has focussed on the dispersal of plants with a fleshy fruit by frugivorous birds, via the transport of seeds within their guts (endozoochory) (Forget *et al.*, 2011). In contrast, much less attention has been paid to dispersal of other plants by waterbirds, despite the diversity of plants likely to be affected (Brochet *et al.*, 2009) and the early interest of Darwin (1859) in this topic.

Migratory waterbirds act as dispersal vectors of wetland plants by ingesting seeds and later egesting them at a different location in a viable condition (Brochet *et al.*, 2009; Van Leeuwen *et al.*, 2012a). Several recent experimental studies have addressed the survival and retention time of different plant seeds passing through the guts of mallards *Anas platyrhynchos* and other dabbling ducks *Anas* spp. (e.g. Brochet *et al.*, 2010; Figuerola *et al.*, 2010; Van Leeuwen *et al.*, 2012a). Such experiments also enable the study of how gut passage affects the subsequent germinability (the probability of germination) and germination time of seeds, and these parameters partly determine probabilities of plant dispersal by endozoochory, and of subsequent establishment. Field studies have demonstrated high rates of seed movement by ducks (e.g. Figuerola, Green & Santamaria, 2003; Green *et al.*, 2008; Brochet *et al.*, 2010b), and modelling has confirmed their capacity to disperse plants over distances of hundreds of km or more (Viana *et al.*, 2013a). Nevertheless, very little research has addressed the role of waterbirds in the expansion of alien plants. Brochet *et al.* (2009) identified many alien plant species whose seeds are present in the diet of migratory ducks in Europe, although they did not demonstrate that these seeds could survive gut passage.

Research on endozoochory of wetland plants to date has mainly been concentrated on dispersal of native plant species by dabbling ducks. Less attention has been paid to the potential that the larger, migratory geese have for plant dispersal, and we are not aware of any previous experimental work. Nevertheless, endozoochory of various plants has been observed for Canada Geese *Branta canadensis* (Neff & Baldwin, 2005), which have been considered responsible for the spread of alien grasses (Isaac-Renton *et al.*, 2010). In Europe, the greylag goose *Anser anser* is an abundant migrant with an extensive range that spreads from Iceland to North Africa.

Viable seeds from *Bolboschoenus* (*Scirpus*) *maritimus* and *S. litoralis* (in Spain, A. J. Green & J. Figuerola, unpubl. data) have been recorded in their faeces. The greylag is increasing in numbers and is now common all year-round in central Europe. Here, we study its potential as a plant vector and compare this with the commonest duck, the mallard.

To study the potential contribution of these differently sized waterfowl to the dispersal of native and alien wetland plants in Europe, we fed mallards and greylag geese with seeds of four plant taxa. Two alien species used were the perennial water primrose *Ludwigia grandiflora* (Onagraceae) and the dense-flowered cordgrass *Spartina densiflora* (Poaceae). The native species were the glaucous glasswort *Arthrocnemum macrostachyum* (Amaranthaceae) and shrubby seablight *Suaeda vera* (Amaranthaceae). Both these salt marsh species are widely distributed around the Mediterranean region and the Canary Islands, and *S. vera* also occurs in Britain (Euro+Med, 2006). Field data confirm that mallards and other dabbling ducks ingest seeds of *Suaeda* and *Arthrocnemum* (Fuentes *et al.*, 2004; Brochet *et al.*, 2009, 2012). Seeds of *Spartina townsendii* and *Ludwigia peploides*, species closely related to *S. densiflora* and *L. grandiflora*, have also been recorded in the diet of mallard and teal *A. crecca* (Brochet *et al.*, 2009, 2012).

The alien species *L. grandiflora* has spread rapidly across central and Northern Europe since its introduction in 1830 to southern France and is classified as a pest organism and one of the most invasive aquatic plant species in Europe (EPPO, 2011; Thouvenot, Haury & Thiebaut, 2013). The ornamental plant trade is considered to be the most important pathway for the introduction of *L. grandiflora* in Europe, and the import and sale of *L. grandiflora* is prohibited in France and Switzerland (EPPO, 2011). Once established, *L. grandiflora* is easily spread by plant fragments with a high regeneration capacity (Hussner, 2009). *Ludwigia grandiflora* produces numerous viable seeds, but there is no previous information about their potential dispersal via waterbirds (EPPO, 2011).

The alien *Spartina densiflora* is invading a variety of estuarine environments around the World (Bortolus, 2006). The first report locating *S. densiflora* outside the Americas was from Spain (Tutin *et al.*, 1980). It was perhaps accidentally introduced to Europe by the lumber trade between South America and Spain in the 16th century (Castillo *et al.*, 2000), although the first botanical records of the species date from the 20th century. It has now expanded along the southern Atlantic coast of the

Iberian Peninsula from the Algarve in Portugal to Algeiras Bay (Bay of Gibraltar) in Spain (Nieva, Castellanos & Figueroa, 2002; Simões *et al.*, 2011). With its tall canopy, dense tussocks and abundant seed production, it outcompetes native plants and invades previously unvegetated salt marshes (Figueroa & Castellanos, 1988). *Spartina densiflora* seeds are very buoyant (Bortolus, Schwindt & Iribarne, 2002) and can be transported long distances in sea water (Morgan & Sytsma, 2013). However, the possibility of dispersal by waterbirds has not previously been addressed. The rate of expansion of this alien since its initial introduction appears to have been much lower than that of *L. grandiflora*.

Seeds of the four plants were fed to mallards and greylag geese to quantify survival after gut passage, retention time, subsequent germinability and germination time. In this study, we consider whether the consequences of gut passage on seed survival and germination are much more variable between different plant species than between bird species, as observed for frugivores (Traveset, Robertson & Rodríguez-Pérez, 2007). We also ask whether larger waterbirds can disperse seeds over a longer maximum distance, due to a longer retention time in the gut, as reported for frugivores (Wotton & Kelly, 2012). These four plant species represent a wide range of seed size (see Figure S1 and Results). It has previously been suggested that species with smaller seeds resist digestion by waterbirds more effectively, with a shorter retention time, but higher retrieval and viability (Soons *et al.*, 2008; Van Leeuwen *et al.*, 2012a). Seeds with a longer retention time are more likely to lose viability before egestion. However, if they remain viable, the time taken to germinate can decrease with longer retention time, as mechanical dormancy is more likely to be broken (Brochet *et al.*, 2010a).

Specifically, the following hypotheses were tested in this study:

- (1). The effects of gut passage on seed retrieval, germinability and time to germination vary between plant species, but not between bird species.
- (2). Alien *L. grandiflora* has greater potential for long-distance dispersal by Anatidae than *S. densiflora*, as suggested by its faster expansion rate in Europe.
- (3). Species with smaller seeds have higher retrieval and germinability, but shorter retention time.
- (4). Owing to larger body size, the retention time of seeds is longer in greylags than in mallards.
- (5). For a given plant species, seeds with a longer retention time have reduced germinability and reduced time taken to germinate.

Methods

Seeds of *A. macrostachyum*, *S. vera* and *S. densiflora* were collected in summer 2011 from various mother plants in natural populations in the Caracoles estate and Lucio del Cangrejo within the Doñana marshlands in southwest Spain. Doñana is the most important wetland complex in Europe and the Western Mediterranean for wintering waterfowl and a major site for seed dispersal by waterfowl (Figuerola *et al.*, 2003; Rendon *et al.*, 2008). *Ludwigia grandiflora* seeds were collected in October 2011 from c. 100 shoots of about 15 plants cultivated in mesocosms at the Heinrich-Heine-University in Düsseldorf, Germany. These plants originated from an invasive population from the Netherlands.

In the laboratory, the seeds were separated, cleaned, dried and stored in plastic vials at ambient external temperature (mean temperature 9.4 °C during the months of January and February 2012) and natural light for at least 2 months at Huelva University. This storage simulates dry periods that are regularly experienced by seed banks in natural Mediterranean wetlands, which reflood in winter. Such drying is particularly typical of temporary salt marshes occupied by *A. macrostachyum* and *S. vera*. To compare the mass and the maximum length of each plant species, 30 seeds per species were dried at 60 °C for 24 h. The seeds were weighed to the nearest 0.1 mg with a precision balance (COBOS A-220-CSI). The maximum length was measured to the nearest 0.01 mm with a COMPECTA digital calliper (ref. 5900601).

Ten adult greylag geese (*Anser anser*, seven females and three males) and 10 adult mallards (*Anas platyrhynchos*, eight females and two males) were used in the experiment. These species have a mean body mass of 3.31 kg and 1.18 kg, respectively (Dunning, 1993). Experimental birds were captive-bred, pinioned and kept in semi-natural conditions at the Cañada de los Pajaros Nature Reserve and avicultural centre (Puebla del Rio, Sevilla, <http://www.canadadelospajaros.com/>), where they were fed principally with wheat grains but supplemented by their own feeding in and around lakes in the reserve. All experimental birds were born between 2008 and 2011, with the exception of one older male goose. During the experiment, they were housed in outdoor facilities at the Cañada de los Pajaros, and fed a constant diet with wheat grains. They were kept individually in metal cages (0.60 × 0.60 × 1.00 m) with a mesh floor and removable plastic trays placed underneath. Wheat grains and water were available *ad libitum* throughout the experiment.

On 20 February 2012, each goose and mallard was force-fed with a mixture of seeds of the four study plants. The mixture included 495 seeds of *A. macrostachyum*, 268 of *S. vera* and 500 of *L. grandiflora*. For *S. densiflora*, an estimated 215 ± 11.23 (mean \pm SE) seeds were given to each goose and 195 ± 16.1 seeds to each mallard (i.e. 9% fewer than geese). The exact number of *S. densiflora* seeds fed to the birds was unknown because, for this species, each spikelet has a flower but not all of them bear fruits (see Figure S1). As seed separation from the spikelet would cause damage, we fed the birds with whole spikelets and estimated the number of seeds inside by mass (after establishing that one seed was present per 9.27 mg of spikelets).

Bird faeces were collected in the removable trays and stored in individual plastic bags at specific time intervals after ingestion: every hour for the first 8 h, then every 2 h up to 48 h after ingestion and finally every 24 h up to 96 h after ingestion. In principle, we expected no retrieval after 72 h or more (but see Results). So as to increase the information on the consequences of gut passage for *S. densiflora* and to compensate for the lower number of seeds of this species ingested, after collecting faeces at 72 h, all birds were then fed with an additional dose of *Spartina* seeds. Geese were fed again with an estimated 300 ± 16.11 seeds and mallard with 280 ± 11.23 seeds. For this reason, for all statistical analyses comparing the retrieval and germination for different plant taxa, only data for the first 72 h were used.

The faeces collected were immediately sieved through a mesh size of 500 μ m to collect intact seeds, which were then classified to species under a binocular microscope, counted and stored dry in separate plastic containers at room temperature (25 °C) until set to germinate. All *S. densiflora* was recovered in faeces as spikelets, and no loose, intact seeds were observed. Spikelets with and without seeds were separated under the binocular microscope.

As non-ingested controls, 100 seeds per taxa were used and stored in the same way prior to germination tests. From all seeds recovered from the birds, a maximum of 20 were used per seed type, individual bird and retention time for germination tests. Hence, for short retention times, when most seeds were retrieved (see Results), not all seeds were set for germination, owing to space limitations. On 22 March 2012, ingested and control seeds were set to germinate in microtitre plates. For *S. densiflora*, only spikelets with seeds were set to germinate. Each cell contained filter paper, distilled water and one individual seed within the spikelet.

Plates were positioned in a germination chamber (CLIMAS, mod. Grow Chamber, AGP1300), with a 12-/12-h light/darkness photoperiod and temperature cycles of 20 °C/8 °C. The number of germinated seeds was determined every 2–3 days.

After 63 days, remaining seeds were removed from the chamber and stored at 4 °C (simulating winter chill). The experiment was thus designed to consider the response of seeds to winter chill and its interaction with gut passage. After 2 months of chilling, seeds were set for a second germination (above conditions). However, only 26 seeds germinated in the second round, compared to 451 in the first (figures including controls). For this reason, results are only presented for data from the first round.

Statistical analyses

Differences in seed mass and length between plant species were tested by ANOVAs with Tukey HSD *post hoc* tests after checking for normality of the data. Data for retrieved and germinated seeds were analysed in four generalised linear mixed models (GLMMs), using likelihood ratio tests to compare models including and excluding terms of interest, and Tukey HSD *post hoc* tests (using the package 'multcomp' in R) to reveal differences between factor levels. All covariates were centred, and the identity of the individual birds was included in all models as a random factor to account for individual differences. All calculations were performed using the package 'lme4' (Bates *et al.*, 2013) in R (R-Development-Core-Team, 2014).

Effects of gut passage on retrieval and germinability

The effects of seed species and bird species on the total proportion of seeds retrieved during the experiment after 72 h were analysed in a first model (GLMM1). The proportion of retrieved seeds was taken as a binomially distributed response variable (with the logit link function), using the number of seeds retrieved as the numerator and the number of seeds not retrieved as the denominator (to account for different numbers of seeds fed). Bird species, propagule species and their interaction were included as factors. In a second model (GLMM2), we tested the effect of gut passage on the proportion of seeds retrieved that germinated. The response variable consisted of the number of germinating seeds as the numerator and the number of seeds not germinating as the denominator in a binomial analysis, thus accounting for differences in the number of seeds

retrieved (or the number of seeds sown for the controls) between groups. Seed treatment (ingested by mallards, ingested by geese or controls) and seed species were included as factors, together with their interaction.

Effect of retention time on seed retrieval and germinability

In a third model (GLMM3a), we tested whether egestion patterns over time were similar between bird species and propagules. The proportion of retrieved seeds at each retention time was taken as a binomially distributed response variable (with the logit link function), using the number of retrieved seeds at that retention time as the numerator and the number of seeds retrieved at other retention times as the denominator (to account for different numbers of seeds fed and different numbers of seeds still remaining in the birds). Retention time was included as a linear as well as a squared covariate, to test for both linear and curvilinear patterns of retrieval. We analysed the data for retention times (RTs) up to 24 h in 2-h intervals (attempts to do this up to 48 h failed because the high proportion of zeros meant that models did not converge). After model selection based on likelihood ratio tests, 'RT²: plant species', 'RT: plant species' and 'RT²: bird species' remained as interactions in the model. In a second similar model (GLMM3b), we analysed the data for retention times up to 72 h, in 24-h intervals in the same way. The same variables were included except for RT² and its interactions, since there were only three retention times.

In the fourth model (GLMM4), the effect of retention time on germinability was analysed, specifically to look at differences between plant and bird species. Whether or not sown seeds germinated was used a binomial response variable, with seed species and bird species as fixed factors, together with linear retention time and RT² as centred covariates, and all the two-way interactions between these terms. After model selection, only the interaction between linear retention time and bird species was excluded from the model. Similar models were also run separately for each plant species to look for specific effects of retention time on germinability within species.

Time to germination

For each plant species, five separate Cox regression analyses were performed using SPSS Statistics 21 (IBM, 2012) to analyse the effects of gut passage on the time taken for seeds to germinate in the first run. In all models, only seeds that germinated were included, using

germination time as the dependent variable (taken as the duration in days before a visible root tip protruded from the seed coat). The five models compared germination time as follows: (i) seeds passed through geese versus controls, (ii) seeds passed through mallard versus controls, (iii) seeds passed through mallard versus geese, (iv) effect of retention time for seeds passed through geese, and (v) effect of retention time for seeds passed through mallard.

Results

Plant species differed significantly in seed mass and length (Table 1; ANOVAs: $P < 0.001$; Figure S1). *Spartina densiflora* spikelets were significantly longer than seeds of the other taxa, whereas *S. densiflora* and *L. grandiflora* seeds were similar in mass and significantly heavier than the other taxa. *Arthrocnemum macrostachyum* was significantly lighter than *S. vera*, but similar in length (Table 1).

Viable seeds of all plant species were retrieved from faeces of both waterfowl species. In total, 2573 intact seeds were collected from mallards (including *S. densiflora* seeds recovered within 72 h, and seeds of other species within 96 h) and 2478 from greylag geese. For mallards, 9.14% of the tested seeds germinated successfully, compared to 24.18% for greylag geese.

Seed retrieval and the effects of gut passage on germinability

The alien *L. grandiflora* had much higher retrieval rates than native taxa, whereas the alien *S. densiflora* had much lower retrieval (Table 2). There was a significant interaction between different plant and bird species in the proportion of seeds that was retrieved over the whole experiment (GLMM1, likelihood ratio test: $\chi^2 = 41.7$, $P < 0.01$). This interaction was due to differences between plant species (Tukey contrasts for 20 of the 24 combinations $P < 0.05$), and there were no differ-

Table 1 Mean mass and length (\pm SE) of the plant seeds used

	Mean mass (in mg)	Mean length (in mm)
<i>Arthrocnemum macrostachyum</i>	0.30 (± 0.02) (a)	1.04 (± 0.03) (a)
<i>Ludwigia grandiflora</i>	1.90 (± 0.07) (b)	2.50 (± 0.05) (b)
<i>Spartina densiflora</i>	1.99 (± 0.09) (b)	9.38 (± 0.18) (c)
<i>Suaeda vera</i>	0.60 (± 0.04) (c)	1.15 (± 0.04) (a)

Species that do not share a common letter differed significantly according to Tukey *post hoc* tests ($P < 0.05$). Measurements for *S. densiflora* refer to spikelets (see Figure S1).

Table 2 Retention times and percentages of retrieved seeds from four plant species for greylag geese and mallard

	Retention time (h)					Retrieval (%)
	Mean	Mode	Median	Maximum		
				I	G	
Geese						
<i>Arthrocnemum macrostachyum</i>	7.2 ± 1.3	3	3	96	72	7.4 ± 2.8
<i>Ludwigia grandiflora</i>	8.9 ± 1.1	3	3	96	72	38.7 ± 5.6
<i>Spartina densiflora</i>	8.2 ± 5.1	3	3	72	2	0.8 ± 0.4
<i>Suaeda vera</i>	16.9 ± 5.3	3	3	96	96	5.6 ± 2.0
Mallards						
<i>A. macrostachyum</i>	4.6 ± 0.8	5	4	72	36	9.7 ± 4.1
<i>L. grandiflora</i>	14.4 ± 4.5	5	5	96	72	35.5 ± 4.3
<i>S. densiflora</i>	5.61 ± 0.9	5	5	72	8	2.2 ± 1.9
<i>S. vera</i>	11.4 ± 2.8	5	5	96	72	9.8 ± 6.8

Seeds retrieved after up to 96 h are included (only up to 72 h for *S. densiflora*). Mean retention time and % retrieval (\pm SE) are calculated from the 10 average values from the 10 individual birds. Retention times are for intact seeds (I) except for maxima, which are also presented for seeds that germinated (G).

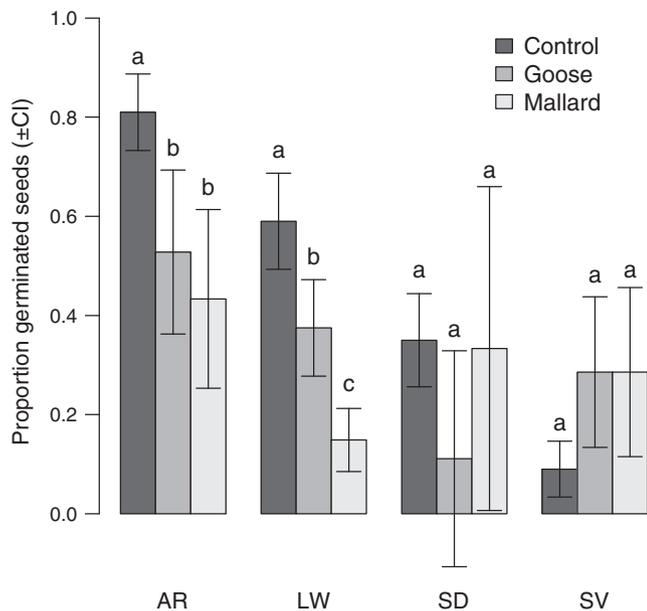


Fig. 1 Proportion of seeds germinating (\pm 95% confidence interval) according to treatment: gut passage through mallard, through greylag geese or controls. AR = *Arthrocnemum macrostachyum*, LW = *Ludwigia grandiflora*, SD = *Spartina densiflora*, SV = *Suaeda vera*. Bars with different letters within plant species differ significantly with $P < 0.05$ in *post hoc* tests from GLMM2 (see Table S1).

ences between bird species for a given plant species (all four comparisons $P > 0.49$).

The germinability of seeds retrieved from birds was highest in *A. macrostachyum*, but only in *S. vera* was it higher than for control seeds (Fig. 1). Gut passage through birds significantly reduced the proportion of seeds germinating compared to controls for *A. macrostachyum* and *L. grandiflora*, with no significant effects for

S. densiflora and *S. vera* (Fig. 1; Table S1). There was only a difference between bird species for *L. grandiflora*, with higher germinability for seeds from geese (Fig. 1; Tukey *post hoc* test, $P < 0.01$; Table S1). The proportion of ingested seeds that were both retrieved and germinated went in the following order: *L. grandiflora* > *A. macrostachyum* > *S. vera* > *S. densiflora* for greylags and *A. macrostachyum* > *L. grandiflora* > *S. vera* > *S. densiflora* for mallards. This difference between bird species reflects the reduced viability of *L. grandiflora* seeds from mallards (Fig. 1).

Retention times of retrieved seeds

Median and modal retention times were very similar for different plant taxa in the same bird species, but consistently longer in mallards than in geese (Table 2; Figure S2). All modal and median retention times were 3 h for geese, but 5 h for mallards (except for a median of 4 h for *A. macrostachyum*). Maximum retention times for retrieved seeds were 72–96 h for all plant species (Table 2). Some seeds of *A. macrostachyum*, *L. grandiflora* and *S. vera* that were recovered after 72 h still germinated, but no seeds of *S. densiflora* retained for longer than 8 h germinated (Table 2). Mean retention time was highest for *S. vera* and lowest for *A. macrostachyum* (Table 2). Overall, 86.5% of seeds retrieved from mallards and 90.6% of seeds retrieved from geese were egested within 12 h (Fig. 2; see also Figure S2).

The longer retention time for mallards was reflected by a significant difference between bird species in the timing of seed retrieval, when analysed by two hourly periods over the first 24 h (significant interaction

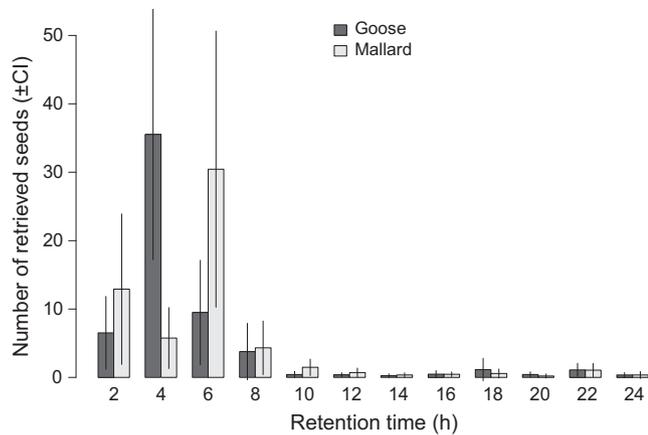


Fig. 2 Mean number of retrieved seeds ($\pm 95\%$ confidence interval) per individual bird from mallard and greylag geese over time for up to 24 h after ingestion. Seeds were retrieved every hour for the first 8 h, but summed to intervals of 2 h in the figure.

between bird species and RT^2 , GLMM3a, $\chi^2 = 48.1$, $P < 0.001$; Fig. 2; Table S2). The peak of retrieval and the decrease in seed egestion over time differed significantly between plant species, as shown by interactions between plant species and RT^2 ($\chi^2 = 293.0$, $P < 0.001$) and (linear) RT ($\chi^2 = 149.4$, $P < 0.001$; Fig. 3a; Table S2). This reflects how mean retention times were highest in *L. grandiflora* and *S. vera* and lowest in *A. macrostachyum* (Table 2).

Further analyses of seed retrieval patterns in 24-h intervals up to 72 h confirmed the differences between bird and plant species, with significant interactions with linear retention time (bird species, $\chi^2 = 5.9$, $P = 0.015$; plant species, $\chi^2 = 18.9$, $P < 0.001$; Table S2). A relatively high proportion of *L. grandiflora* seeds was retrieved after 14–96 h in the digestive system (Figs 3 and S2).

Effects of retention time on germinability

Changes in seed germinability with time retained in the gut were specific to different plant and bird species, with distinct curvilinear effects (Fig. 4) and significant interactions between linear or squared retention times and plant or bird species in GLMM4 ($P < 0.05$; Table S2). *Post hoc* tests showed that the relationship between germinability and retention time differed between *L. grandiflora* and *A. macrostachyum* both in linear ($P < 0.02$) and squared retention time terms ($P < 0.05$). This reflected less viability for *L. grandiflora* when retained for a long time. *Spartina densiflora* and *S. vera* did not differ significantly from other plant species. After removing all interactions, retention time had a significantly negative main effect on seed germinability ($P < 0.01$).

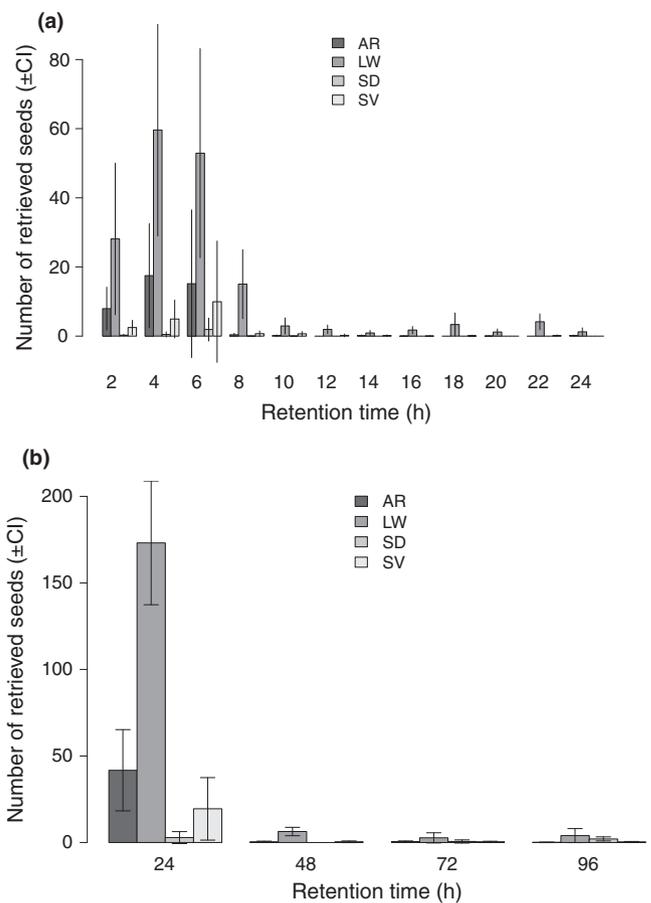


Fig. 3 Number of seeds retrieved per bird ($\pm 95\%$ confidence interval) for each plant species over time (a) in 2-h intervals up to 24 h after ingestion. (b) in 24 h intervals. AR = *Arthrocnemum macrostachyum*, LW = *Ludwigia grandiflora*, SD = *Spartina densiflora*, SV = *Suaeda vera*. Additional *S. densiflora* seeds were ingested after 72 h (see Methods).

When equivalent GLMMs were computed separately for each plant species, there were only significant effects of retention time for *L. grandiflora*. For the *L. grandiflora* model, there were significant interactions between bird species and both linear and squared retention times (Table S2). As retention time increased, the viability of *L. grandiflora* seeds decreased in both bird species, but was always lower in mallards (Fig. 4b).

Time to germination and its relationship with retention time

Gut passage had a strong effect on the time taken by *A. macrostachyum*, *L. grandiflora* and *S. densiflora* seeds to germinate in the first germination run (Table 3; Figs 5 & 6). Germination of *A. macrostachyum* and *S. densiflora* was accelerated by gut passage (Table 3; Fig. 5). The effect of gut passage on *L. grandiflora* depended strongly

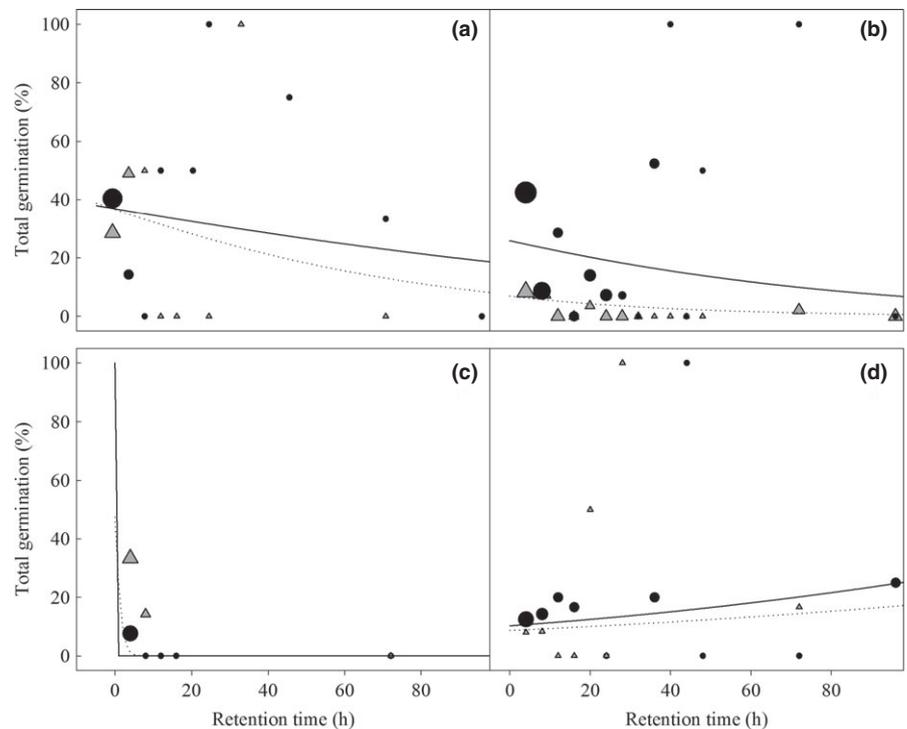


Fig. 4 Proportion of seeds germinated for (a) *Arthrocnemum macrostachyum*, (b) *Ludwigia grandiflora*, (c) *Spartina densiflora* and (d) *Suaeda vera*, according to the retention time in greylag geese (black circles and solid line) and mallard (grey triangles and dotted line). The size of symbols is proportional to the number of seeds tested for germination. Regression lines were fitted in STATISTICA 8.0 software with a binomial error distribution and LOGIT link function (applied to raw data with 0 for seeds that did not germinate and 1 for those that did).

Table 3 Cox regression of the effects of gut passage and retention time on time to germination. Significant *P* values are shown in bold

Plant species	Cox regression test	χ^2	d.f.	<i>P</i> -value
<i>Arthrocnemum macrostachyum</i>	(1) Geese versus Control	19.838	1	<0.001
	(2) Mallards versus Control	22.075	1	<0.001
	(3) Geese versus Mallards	0.168	1	0.682
	(4) Geese Retention Time	11.433	14	0.652
	(5) Mallard Retention time	12.046	14	0.603
<i>Ludwigia grandiflora</i>	(1) Geese versus Control	56.565	1	<0.001
	(2) Mallards versus Control	272.578	1	<0.001
	(3) Geese versus Mallards	84.784	1	<0.001
	(4) Geese Retention Time	143.373	25	<0.001
	(5) Mallard Retention Time	56.831	27	<0.001
<i>Spartina densiflora</i>	(1) Geese versus Control	9.029	1	0.003
	(2) Mallards versus Control	9.097	1	0.003
	(3) Geese versus Mallards	0.055	1	0.815
	(4) Geese Retention Time	3.250	5	0.662
	(5) Mallard Retention Time	19.053	5	0.002
<i>Suaeda vera</i>	(1) Geese versus Control	1.249	1	0.264
	(2) Mallards versus Control	0.372	1	0.542
	(3) Geese versus Mallards	0.220	1	0.639
	(4) Geese Retention Time	16.227	18	0.577
	(5) Mallard Retention Time	19.311	13	0.114

on retention time, with a delay in germination after a longer time in the gut, an effect also detected for *S. densiflora* in mallards (Table 3; Fig. 6). A difference between bird species was detected only for *L. grandiflora*, with germination generally faster in seeds fed to mallards (Table 3; Figs 5 and 6). The variability in time to germination of *S. vera* seeds was increased after gut passage (Fig. 5).

Discussion

Waterfowl clearly have a considerable capacity to disperse both native and alien plants, including invasive taxa such as *Ludwigia* and *Spartina*. Seeds can be retained in the gut for periods of 3 days or more before egestion in a viable condition, and we show that geese can be major vectors as well as ducks. This study

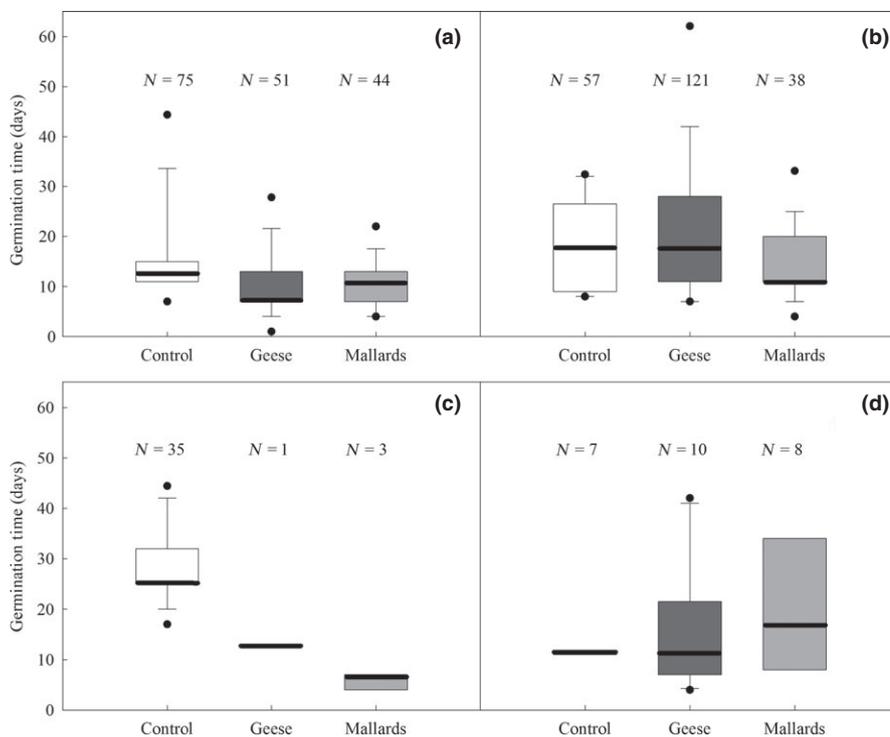


Fig. 5 Box plots of germination time for (a) *Arthrocnemum macrostachyum*, (b) *Ludwigia grandiflora*, (c) *Spartina densiflora* and (d) *Suaeda vera* for control (white), greylag geese (dark grey) and mallard (light grey). The lower and upper boundaries of the box mark the 25th and 75th percentiles, a line within the box marks the median, whiskers (error bars) indicate the 90th and 10th percentiles, and black dots are outliers.

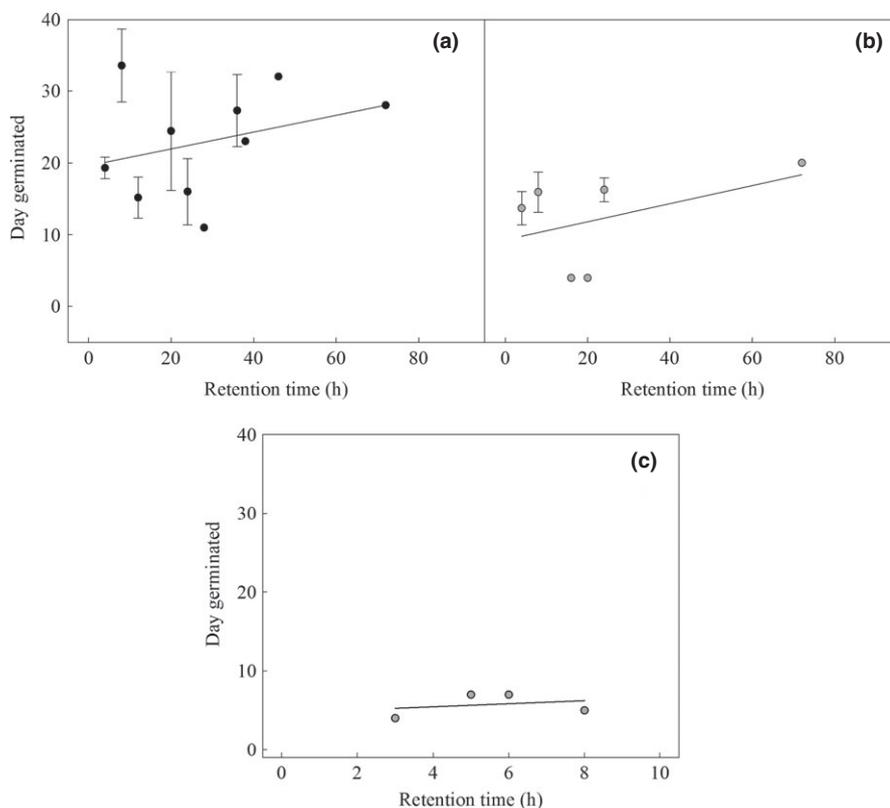


Fig. 6 Day of germination (\pm SE) of (a, b) *Ludwigia grandiflora* and (c) *Spartina densiflora* against retention time in greylag geese (solid black circles) and mallards (grey filled circles). Ordinary least squares regression lines were fitted in SIGMAPLOT 11.0 (Systat Software, 2006).

underlines the great capacity of geese and ducks to disperse seeds of plants lacking a fleshy fruit. In the case of native plants, this represents a major ecosystem service provided by these birds (Green & Elmberg, 2014). The

importance of these birds as vectors of alien plants has been consistently overlooked (Brochet *et al.*, 2009), an oversight that will limit the success of efforts to manage the spread and impact of these species.

Most experimental studies of internal transport by waterfowl over the past three decades have been run for short periods of 48 h (e.g. Soons *et al.*, 2008; Wongsriphuek, Dugger & Bartuszevige, 2008; Brochet *et al.*, 2010a), whereas we were still retrieving some viable seeds when our experiment finished at 96 h. This suggests that the maximum retention time (at least under captive conditions) and thus the maximum distance for long-distance dispersal have often been underestimated in previous studies. For example, Viana *et al.* (2013a) combined data on migratory movements and retention time and showed great potential for dispersal of *Scirpus* seeds by mallard and teal in Europe and North America, with maximum dispersal distances of >1000 km, but they relied on feeding experiments that ended after 48–56 h. Our study suggests that even longer distance dispersal might be possible. Furthermore, De Vlaming & Proctor (1968) recorded similarly long maximum retention times in mallard for plants such as *Potamogeton pectinatus* (73 h) and *Eleocharis* spp. (93 h).

This first experimental study to include geese confirms that, like ducks, migratory geese also have a major capacity as plant vectors. Field studies on other geese species suggest they can disperse a wide range of plant species (Neff & Baldwin, 2005; Bruun, Lundgren & Philipp, 2008). Their role as vectors is likely to be particularly important at extreme latitudes (e.g. in the Arctic) where many geese species breed and where rapid climate change is shifting the distribution of suitable habitat for plants. At lower latitudes (e.g. central and southern Europe), geese may be less important than the more abundant ducks, although geese may be more likely to disperse emergent or terrestrial plant seeds when grazing out of the water.

Consequences for plant invasions

Brochet *et al.* (2009) showed that seeds of many alien plants (both aquatic and terrestrial) are recorded in the diet of migratory ducks in Europe. Viable propagules of various alien plants have also been isolated from waterfowl faeces (Green *et al.*, 2008; Brochet *et al.*, 2010b), confirming that such dispersal occurs in the field. We confirmed the potential of two invasive plants to disperse by endozoochory, especially *L. grandiflora* which is a major threat to aquatic plant assemblages in Mediterranean and European wetlands, due to its shading effects on waterbodies (Stiers *et al.*, 2011) and its allelopathic effects that reduce survival of other species (Dandelot *et al.*, 2008).

Our results suggest *Ludwigia* spp. (Onagraceae) have a particularly strong capacity to disperse within waterbird guts. Most of the 82 *Ludwigia* species are from tropical and subtropical regions, where there have been no studies of plant dispersal by waterbirds. Closely related species tend to be similar in their ability to resist gut passage (De Vlaming & Proctor, 1968), and it is noteworthy that other *Ludwigia* species are invasive (e.g. *L. peruviana* and *L. longifolia* in Australia; Chandrasena, 2005). In particular, *L. peploides* is highly invasive in Europe and is regularly ingested by migratory ducks (Brochet *et al.*, 2012).

Despite confirming the potential for internal dispersal by migratory waterfowl for both alien plants, our results suggest higher potential for *L. grandiflora* than for *S. densiflora*, since a much higher proportion of *L. grandiflora* seeds were retrieved and germinated and they were retained in the gut for longer. This is consistent with a much greater expansion of *L. grandiflora* within Europe since it was first detected than is the case for *S. densiflora*.

New populations of *L. grandiflora* recently became established in the Netherlands and Germany, far away from the closest known invaded sites in France, indicating only human or waterbird vectors could be responsible. Unlike many other introduced aquatic plants, *L. grandiflora* is not common in German trade (Hussner, Nehring & Hilt, 2014), reducing the probability of an introduction by humans. Furthermore, the new population in Northern Germany is in an area rarely visited by people, but frequented by migratory greylag geese (D. Kolthoff pers. comm.).

Our study has only focussed on internal transport, but external transport (epizoochory) may also be an important dispersal mode for invasive plants (Figuerola & Green, 2002; Coughlan, Kelly & Jansen, 2015). Diaspores of smooth cordgrass *Spartina alterniflora*, a highly invasive species in many parts of the world, were found on feet and feathers of brant geese *Branta bernicla* and on three duck species in New Jersey (Vivian-Smith & Stiles, 1994). Coots (*Fulica atra*) also make extensive use of *L. grandiflora* stems to make their nests in invaded sites and, because these stems act as vegetative propagules and often survive to flower in the nests (pers. obs.), this may aid dispersal of this alien plant at a local scale. Although external transport should also receive more attention in future, internal transport by waterfowl is generally much more frequent than external transport (Brochet *et al.*, 2010b).

Body size and retention time

To our knowledge, this is the first experimental study comparing Anatidae species to find a clear interspecific

difference in seed retention patterns. This may be partly due to our relatively large sample size (10 individuals per species) and the greater difference in body size than that recorded between the dabbling ducks used in some previous studies. For instance, three-five individuals of each Anatidae species were used by Charalambidou, Santamaria & Langevoord (2003) and by Figuerola *et al.* (2010).

Our results do not support the third hypothesis that bigger waterfowl retain seeds for longer, and we found the opposite to the positive scaling relationship between body size and retention time recorded in frugivorous birds (Wotton & Kelly, 2012). Because larger birds have longer guts, it may seem counterintuitive that seeds were retained for longer in smaller waterbirds. However, our finding is in line with the comparative analysis by Viana *et al.* (2013b), who combined data from earlier studies and found the median retention time to be negatively correlated with body size. Hence, a negative relationship between body size and retention time may be a general pattern in waterbirds.

Two possible explanations for our results are worth investigating in the future. Firstly, seeds are likely to be retained for longer in the gizzard of the largely granivorous mallard than in that of the largely herbivorous greylag, as geese have longer intestines required for digestion of green plant material. Secondly, it may be harder for seeds (especially larger ones) to pass the sphincter that separates the gizzard from the intestine in the smaller mallard. The much longer mean retention of *L. grandiflora* in mallards, and the particularly high number of *L. grandiflora* seeds retained for more than 48 h (Table 2; Figure S2), is consistent with this proposal.

Differences between plant species in seed retrieval and retention time

The particularly strong capacity for endozoochory in *L. grandiflora*, which has the largest seeds of the four plant species studied, does not support our initial hypothesis that smaller seeds would have greater dispersal potential. Previous authors (e.g. Soons *et al.*, 2008) have argued that size reduces the chances of long-distance dispersal because large seeds are more likely to be retained and destroyed in the waterfowl gizzard. In a meta-analysis, Van Leeuwen *et al.* (2012a) found that larger propagules (including plant seeds) have lower survival during passage through the waterfowl gut. Our findings for *L. grandiflora*, a large seed which showed the highest retrieval and has a relatively thick seed coat (pers. obs.), demonstrate that there are plant species

with larger seeds which resist digestion. Our results suggest that other factors, such as seed fibre content (Wongsriphuek *et al.*, 2008) or water impermeability (D'hondt & Hoffmann, 2011), may be important additional predictors of seed survival and can override a general trend for large seeds to survive less well.

Our results for *L. grandiflora* suggest that large, hard seeds can be those with the longest retention times in waterfowl: larger seeds will have longer retentions, but only if they are relatively resistant to digestion (see also Van Leeuwen, Tollenaar & Klaassen, 2012b). In contrast, the lightest seed *Arthrocnemum* had the shortest retention time. The *Spartina* seeds were retained relatively briefly, despite their length, probably because they were readily digested. The low retrieval and brief retention of *S. densiflora* may be related to the structure of this seed, as the soft and long, narrow spikelets may have low resistance to the grinding activity in the gizzard.

Effects of gut passage on germinability and germination time

We found greater variation in the response to gut passage between plant species than between waterfowl species, supporting our initial hypothesis. The effects of the two bird species were generally consistent, except for a stronger reduction in the germinability of *L. grandiflora* seeds by mallard than by greylag geese (perhaps related to longer retention in the gizzard). These results are consistent with previous experimental waterfowl studies, which have found a wide variation in germinability and germination time responses between plant species fed to a single duck species (e.g. Soons *et al.*, 2008; Wongsriphuek *et al.*, 2008; Brochet *et al.*, 2010a), but less variation in the effects of different duck species (Charalambidou *et al.*, 2003; Pollux, Santamaria & Ouborg, 2005; Figuerola *et al.*, 2010).

The effect of gut passage on seeds is clearly related to the time that a seed is retained within the gut. Germinability of many wetland plant species has been found to reduce steadily with prolonged retention in dabbling ducks (Pollux *et al.*, 2005; Wongsriphuek *et al.*, 2008), although the exact form of the curvilinear relationship between time and germinability varied between plant species (Brochet *et al.*, 2010a). The reduction in germinability at the highest retention times, especially in *L. grandiflora*, is consistent with previous studies. However, we found significant differences between plant and bird species in the relationship between germinability and retention time, and an especially strong decline in germinability with increasing time for *L. grandiflora* com-

pared to *A. macrostachyum* (Fig. 4). The differences between these two species were the only ones that were statistically significant, probably because of greater statistical power, since more seeds of these two species were recovered and a higher proportion germinated (Table 2). Germinability of *L. grandiflora* declined more strongly at the longest retention times in the mallard, perhaps because this species is more granivorous than the greylag goose.

In addition to influencing the probability that a seed germinates at all (germinability), passage through the gut can also affect the time it takes seeds to germinate. Germination time decreased consistently with increasing retention for a range of plant species studied by Brochet *et al.* (2010a). For the alien species *L. grandiflora* and *S. densiflora*, we found the opposite trend, contrary to our initial hypothesis. This and previous studies (Figueroa *et al.*, 2010) together show that there is a high degree of variability in the effect of retention on the germination time of ingested seeds. In an evolutionary context, whether such earlier or later germination represents any fitness advantages is unclear and will depend on the conditions for plant establishment, including the activity of herbivorous waterbirds (Figueroa & Green, 2004; Figueroa *et al.*, 2005).

Future research should aim to determine which seed traits explain the plant species-specific effects of gut passage on germination capacity of wetland plants. Effects of ingestion may be due to differences in seed coat thickness and texture, seed hardness and permeability, seed age or secondary effects derived from natural levels of seed dormancy (Traveset *et al.*, 2007). This subject remains, as yet, largely unexplored for endozoochory of wetland plants by waterbirds.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Statistical details corresponding to Fig. 3.

Table S2. Likelihood Ratio Tests corresponding to GLMMs 3 and 4.

Figure S1. Seeds of *A. macrostachyum*, *L. grandiflora*, *S. vera* and *S. densiflora*.

Figure S2. Details of seed retrieval over time.

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