ISSN 0028-1042, Volume 97, Number 6

Naturwissenschaften (2010) 97:555–565
DOI 10.1007/s00114-010-0671-1

ORIGINAL PAPER

Internal dispersal of seeds by waterfowl: effect of seed size on gut passage time and germination patterns

Jordi Figuerola · Iris Charalambidou · Luis Santamaria · Andy J. Green

Received: 17 February 2010 / Revised: 12 April 2010 / Accepted: 15 April 2010 / Published online: 29 April 2010

Abstract Long distance dispersal may have important consequences for gene flow and community structure. The dispersal of many plants depends on transport by vertebrate seed dispersers. The shapes of seed shadows produced by vertebrates depend both on movement patterns of the dispersers and on the dynamics and effects of passage through the disperser's gut (i.e. the retention time, survival and germination of ingested seeds). A combination of experiments with captive waterbirds and aquatic plant seeds was used to analyse the following: (a) the effects of inter- and intra-specific variation in seed size and duck species on seed retention time in the gut and (b) the relationship between retention time and the percent germination and germination rates of seeds. Among the three *Scirpus* species used, those with smaller seeds showed higher survival after ingestion by birds and longer retention times inside their guts than those with larger seeds. For *Potamogeton pectinatus*, only seeds from the smaller size class (<8 mg) survived ingestion. Retention time affected the percent germination and germination rate of *Scirpus* seeds but in a manner that varied for the different plant and bird species studied. We recorded both linear and non-linear effects of retention time on percent germination. In addition, germination rate was positively correlated with retention time in *Scirpus litoralis* but negatively correlated in *Scirpus lacustris*. Small seed size can favour dispersal over larger distances. However, the effects of retention time on percent germination can modify the seed shadows produced by birds due to higher percent germination of seeds retained for short or intermediate periods. The changes in dispersal quality associated with dispersal distance (which is expected to be positively related to retention time) will affect the probability of seedling establishment over longer distances and, thus, the spatial characteristics of the effective seed shadow.

Keywords Dispersal quality · Effects of seed ingestion by vertebrates · Endozoochory · Germination rate · Seed dispersal · Effective seed shadow · Seed size

Introduction

Dispersal of seeds has important impacts on gene flow and community structure. The colonisation process and population structure of many organisms can only be understood if we account for low frequency but highly important cases of long distance dispersal (Nathan et al. 2008). Both vector and seed characteristics are likely to influence the potential for long distance dispersal (Nathan et al. 2008).

J. Figuerola (✉) · A. J. Green
Department of Wetland Ecology,
Estación Biológica de Doñana, CSIC,
Apartado de Correos 1056,
E-41080 Sevilla, Spain
e-mail: jordi@ebd.csic.es

I. Charalambidou · L. Santamaria
Netherlands Institute of Ecology-KNAW,
P.O. Box 1299, 3600 BG Maarssen, The Netherlands

Present Address:
I. Charalambidou
Unit of Environmental Studies, CCEIA,
University of Nicosia (Intercollege),
46 Makedonitissas Avenue,
Nicosia 1700, Cyprus

Present Address:
L. Santamaria
IMEDEA, CSIC-UIB,
c/Miquel Marquès 21,
E-07190 Esporles, Mallorca, Spain

The relationship between seed size and the quantity (i.e. number of dispersed seeds) and quality (i.e. fitness of dispersed compared to non-dispersed seeds) components of dispersal by vertebrates remains poorly understood (Schupp 1993), particularly for plant species that do not produce fleshy fruits. Seed mass is assumed to have evolved as a compromise between contrasting selective pressures. On the one hand, large seeds provide plant species with a higher capacity to survive adverse conditions such as shade, drought and competition and, thus, increase establishment capacity (Westoby et al. 1992, 1996; Geritz 1995; Turnbull et al. 1999). On the other hand, higher plant fecundities are associated with production of smaller seeds due to a trade-off between seed quantity and size (Smith and Fretwell 1974; Turnbull et al. 1999).

Small seeds also have higher dispersal capacities since they are more readily dispersed (Smith and Fretwell 1974; Jakobsson and Eriksson 2000). Seed size can influence the probability of consumption by vertebrates (Jansen et al. 2002), the capacity to pass undamaged through their gut (Soons et al. 2008) and the time seeds remain inside them (i.e. their *retention time*; Gardener et al. 1993). In the case of aquatic plants, hydrochory is an important vector of dispersal for many species and may exert strong selection on seed morphology (i.e. acting on traits related to seed buoyancy; Pollux et al. 2009). However, long distance dispersal to non-hydrologically connected water-bodies is restricted to wind and animal-mediated dispersal. Internal transport by ducks and coot is particularly important (Brochet et al. 2009, 2010).

Dispersal efficiency is affected by disperser morphology, in particular, the morphology of the digestive tract (Jordano 2000). For example, waterbirds with smaller gizzards dispersed more *Ruppia maritima* L. (Wigeongrass) seeds because they destroyed a smaller fraction of ingested seeds (Figuerola et al. 2002). In addition, grit abundance was positively correlated with the proportion of *R. maritima* seeds germinating following gut passage (Figuerola et al. 2002), probably due to the scarification effect of grit that facilitated seed germination. Disperser body size and intestine length have been proposed to affect retention time and, consequently, the quality of dispersal (Karasov 1990; Traveset 1998; Jordano 2000; but see Figuerola et al. 2002). Differences in the germination patterns of seeds ingested by different birds have often been attributed to differences in retention time (Barnea et al. 1991; Murphy et al. 1993). Longer retention times can result in dispersal over larger distances and thus favour extended gene flow and colonisation capacity, unless counteracted by a negative effect of passage time on seed germination. Indeed, Charalambidou et al. (2003) and Wongsriphuek et al. (2008)) reported negative effects of retention time on the germination percentage of seeds from, respectively, *R. maritima* fed to five duck species and four (out of seven) plant species fed to Mallards. Consequently, any effect of retention time on germination would influence the size and shape of effective seed shadows (the spatial distribution of viable seeds) and would suggest that predictions of seed shadows solely on the basis of bird movements and rates of gut passage (Westcott and Graham 2000) may be misleading estimations of the distribution of viable seeds.

We tested this hypothesis by comparing the germination patterns of seeds recovered after different retention times in the gut of a given disperser. For this purpose, we performed three experiments that tested the effects of seed size and retention time inside bird guts on three qualitative components of dispersal effectiveness, namely (a) the capacity to survive ingestion, (b) the capacity to germinate (percent germination) and (c) the germination rate (the time span between seed defecation and germination). The first two experiments examined the effect of inter-specific variation in seed size, while the third focused on the effect of intra-specific variation in seed size.

Materials and methods

Experiment 1 We analysed the relationship between seed size and retention time after ingestion by Mallard (*Anas platyrhynchos*) and how retention time affected the germination patterns of ingested seeds (both the proportion of seeds germinating and rate of germination). Fresh mature fruits of *Scirpus lacustris* L. (Bulrush) with mean size 2.2× 1.5×1.0 mm (Campredon et al. 1982) and *Scirpus maritimus* L. (Alkali Bulrush; 3.1×2.2×1.2 mm) were collected in October 1998 from a littoral stand at Lake Lauwersmeer (The Netherlands). Individual seeds were separated from the achenes and stored dry at 4°C. Six captive Mallard, three males and three females, were used in this experiment. They were housed in outdoor facilities at the Netherlands Institute of Ecology in Heteren and fed a constant stable diet of commercial pellet and mixed grains. During the experiment, they were kept individually in wooden cages (0.60×0.50×0.50 m) with a mesh floor (mesh size 12 mm) and removable plastic trays placed under each cage. Mixed grains and water were available ad libitum throughout the experiment (see Charalambidou et al. 2003 for more details).

On 18 August 1999, each Mallard was force-fed with 300 seeds each of *S. lacustris* and *S. maritimus*. To facilitate force-feeding, the seeds were mixed with food pellets soaked in water and formed into oblong pill-shaped pellets. Duck faeces were collected in the removable trays at specific time intervals after ingestion: every hour for the first 4 h, then every 2 h up to 8 h after ingestion (i.e. at 6 and 8 h) and finally every 4 h up to 48 h after ingestion.

Collected faeces were immediately sieved (sieve size 150 µm) to separate the intact seeds, which were then classified to species by visual inspection (under a binocular, whenever necessary), counted and stored in separate closed plastic containers filled with tap water and placed at 4°C until they were set to germinate.

On 18, 19 and 25 August 1999, ingested and control seeds were set to germinate in microtiter trays. Each cell was half filled with tap water, and individual samples were placed in separate cells with a maximum number of ten seeds per cell. Trays were positioned in a light chamber at 20–25°C in daytime, 5–10°C at night and a photoperiod of 12-h light/12-h dark. These conditions result in the optimal germination of both *Scirpus* species (Clevering 1995). The number of germinated seeds was recorded every 7 days. Seeds infected by fungi were considered non-viable and removed to avoid contamination of remaining seeds. After 60 days (i.e. when no additional germination was observed for at least 2 weeks), the remaining seeds were removed from the incubator and stored at 4°C (simulating winter stratification typical of the locality of origin). After 11 months of stratification, the seeds were set for a second germination run (same conditions as above from 13 September 2000 to 21 January 2001) that was terminated 130 days later. Extensive stratification aimed at ensuring that total germination (hereafter referred to as "percent germination") approached seed viability (Clevering 1995, Santamaría et al. 2002). However, we cannot exclude that some seeds remained in dormancy after the second germination trial. Hence, percent germination may underestimate real seed viability. In all three experiments, we did not assess viability by means of tetrazolium staining because we failed to obtain reliable and repeatable results in preliminary tests using non-ingested seeds.

Experiment 2 We expanded the test performed in the first experiment and compared the resistance of seeds of two *Scirpus* species to ingestion by four waterbird species. Survival and germination patterns of ingested seeds were compared among seed and bird species.

The second and third experiments were performed at the Wildlife Recovery Centre in Doñana National Park, southwest (SW) Spain. Most waterbirds were wild animals captured in the field when injured or sick but that had fully recovered and been housed in the centre for several months before the start of the experiment. All Marbled Teal individuals were captive breed. During the experiments, birds were individually housed in pens (3-m long by 3-m wide), with wire mesh covering the sides and roof. The pens contained a rectangular concrete pond surrounded by a band of soil 0.5-m wide that was covered with a layer of fine sand. Prior to the start of the experiments, the ponds were emptied and allowed to dry, and the drainage pipe was sealed with plastic bags and adhesive tape. Two of the pens lacked a concrete pond, and the entire floor was covered with fine sand. The birds were randomly distributed between pens (one to a pen) before the start of the experiments and allowed to acclimate overnight. Water and food (commercial duck-food pellets) were provided ad libitum on separate dishes.

Fresh mature fruits of *Scirpus litoralis* Kuntze (Bulrush; 1.6×1.3×0.7 mm) and *S. maritimus* were collected in October 1998 in the Caño de Guadiamar (Doñana National Park marshes, Sevilla, SW Spain). They were stored dry at room temperature. Sixteen waterfowl were used in this experiment: three Eurasian Teal (*Anas crecca*), five Common Coot (*Fulica atra*), five Marbled Teal (*Marmaronetta angustirostris*) and three Red-crested Pochard (*Netta rufina*). On 11 June 1999, each bird was force-fed with 300 seeds each of *S. maritimus* and *S. litoralis* using the same technique as in experiment 1. Faeces were collected from the concrete pools and sand floor of the enclosure while the birds were present, but the birds had become accustomed to human presence during the months preceding the start of the experiment and showed no signs of stress. Faeces were collected 2 and 4 h after force-feeding and then every 4 h up to 56 h. Faeces were immediately sieved (sieve size 250 µm), and intact seeds were classified to species by eye (as above), counted and stored in separate closed plastic containers filled with tap water and placed at 4°C. On 20 July 1999, the ingested and control seeds were randomly distributed in microtiter trays (as above) and placed in a growth chamber under the same conditions used in experiment 1. Germination was checked every 2 days for 4 months and then every week for one additional month. After 5 months (on 20 December 1999), seeds that had not germinated during this period were stratified for 9 months at 4°C to make sure that germinability approached viability (as above). Subsequently, they were set for a second germination run that lasted 130 days (from 13 September 2000 to 21 January 2001).

Experiment 3 We investigated whether intra-specific variation in seed size affects dispersal quality provided by ducks. Fresh seeds of *Potamogeton pectinatus* L. (Fennel Pondweed) were collected in Veta la Palma (Doñana National Park) in October 1998 and stored at 4°C. On 2 July 1999, ten captive breed Marbled Teal were each force-fed a mixture of 40 seeds with a diameter less than 2.2 mm and 40 seeds with a diameter larger than 3.3 mm. These seed limits were chosen to allow for the visual discrimination of seed size class in seeds recovered from faeces. Duck faeces were collected at the same time intervals and processed similarly to experiment 2. Retrieved seeds were assigned to size classes using their fresh weights after removing the exocarp (<2.2 mm, range 3.2–7.8 mg;

>3.3 mm, range 9.9–14.6 mg). On 22 September 1999, the ingested and control seeds were randomly distributed in microtiter trays (as above) and placed in a growth chamber (at 20°C and a photoperiod of 12-h light–12-h dark; Santamaría et al. 2002). Germination was checked every 2 days. After 77 days (7 December 1999), seeds that had not germinated were stratified for 3 months at 4°C and tested again for germination for a period of 70 days (from 7 March 2000 to 16 May 2000).

Statistical analyses

Effects of seed ingestion by birds were analysed using three parameters. Seed survival was estimated as the number of intact seeds (i.e. those that retained an intact seed coat) extracted from bird faeces. Consequently, seed survival reflects the mechanical damage received by the seeds during gut passage but is insufficient to detect effects of chemical damage on seed viability—which will instead be measured as decreases in percent germination in the subsequent germination run. Percent germination was estimated as the proportion of seeds that germinated by the end of the two germination trials. This variable was considered a surrogate of seed viability, owing to the use of extended stratification periods between the first and second germination trials (Clevering 1995; Santamaría et al. 2002). Germination rate was related to the time elapsed from the start of the first germination trial to each germination event (i.e. for each individual seed and defined as the moment when a visible root tip was observed to protrude from the seed coat). Hence, smaller values of this "germination time" corresponded to faster germination rates, i.e. to an early start of germination. All seeds that germinated on the second germination trial were included as censored data (the germination occurred after the end of the first trial) in the germination rate analyses.

The total number of intact seeds surviving ingestion was compared using paired *t* tests (experiments 1 and 3) or generalised linear models (GLMs) with two factors (waterbird species and seed species) and their interaction (experiment 2). In the latter case, a repeated within-subject effect (i.e. a random effect) coding for bird individual was included to correspond with paired *t* tests for multiple factors. A negative binomial error structure and log link function were used to fit the model, owing to the nature of the response variable (number of defecated seeds, see Figuerola et al. 2002), using the GENMOD procedure of SAS v8.2 (SAS Institute 2000). Count data tend to be distributed according to a Poisson distribution. However, the Poisson distribution assumes that the occurrence of one observation does not affect the probability of further observations. This is not the case in our data because the abundance of seeds in the faeces followed a clumped distribution (i.e. when a seed is present in a faecal sample, the probability of more seeds being present in that sample increases). In statistical terms, this corresponds to variances larger than the means (in the Poisson distribution, mean and variance have the same value). For this type of data, the negative binomial distribution provides a better alternative, by relaxing the assumption of independence between occurrences in a given sample (see SAS Institute 2000).

The effects of seed species (experiments 1 and 2) and bird species (experiment 2) on seed retention time (the number of hours elapsed between force-feeding and appearance of the seeds in the dropping samples) were analysed with GLMs, using a gamma error distribution and inverse link function. Retention time was introduced as a continuous independent variable, and the identity of individual birds was controlled as a repeated subject effect with a random intercept using the macro GLIMMIX for SAS v8.2 (SAS Institute 2000). Residual degrees of freedom were computed by dividing them into between-subject and within-subject portions with the BETWITHIN option.

Percent germination of seeds was analysed in independent analyses for each plant species using mixed model GLMs (with macro GLIMMIX) with a binomial error distribution and a logit link function. The number of germinated seeds was used as the numerator in the dependent variable and the total number of seeds in the germination trials as the denominator. First, we tested the effect of ingestion by birds by considering seed ingestion factor (ingested vs control). For the third experiment, we analysed the differences in percent germination between seed size groups. The effects of retention time (experiments 1 and 2) and bird species (experiment 2) were analysed in separate GLMs for each plant species as in the previous analyses, but control (non-ingested) seeds were excluded. Since the effects of retention time are not necessarily linear, both quadratic and cubic factors of retention time were also tested in the initial models. Model selection followed a backward removal procedure, starting with an initial model including retention time as a third order polynomial. For experiment 2, the initial model also included bird species (as a fixed factor) and the interaction between bird species and the linear term for retention time. A random factor to control for the effects of bird individual was included in the analyses.

The results of passage time and percent germination of seeds according to passage time were combined by plotting the number of seeds defecated at each time interval and the number of these seeds that successfully germinated, as estimated from mixed model GLM parameter estimates.

The effect of waterbird gut passage (ingested vs control seeds) and seed retention time (only for ingested seeds) on the rate of seed germination were analysed by fitting separate Cox proportional hazards regression models (e.g. Allison 1995) to data consisting of the number of days between initiation of germination trials and seedling emergence, for each individual seed. Only data from seeds that germinated by the end of both germination trials were included to separate the effects of ingestion on the rate of germination from the effects on germinability. Seeds that germinated in the second round of germination were coded as "right censored" because germination occurred after the end of the first germination trials of 60 (experiment 1) or 150 days (experiment 2), see Therneau and Grambsch (2000). To account for the effects of ingestion by different individuals, a replicate effect was added to both models as a random or "frailty" effect (equivalent to the random factor included in previous analyses using GLMs). Seed ingestion (ingested vs control) and retention time were entered in separate analyses as fixed effects, which were tested using the EM algorithm (Therneau and Grambsch 2000). Separate models were constructed for each of the two seed species in experiments 1 and 2. In experiment 2, the model analysing retention time also included the factor "bird species" and its interaction with retention time. Ties were dealt with using the Efron method (Therneau and Grambsch 2000). Survival analyses were computed using S-Plus 2000 (Mathsoft 1999). As for the analyses of percent germination, we followed a backward removal model selection procedure starting with an initial model including retention time as a third-order polynomial. Again, for experiment 2, the initial model also included bird species and the interaction between bird species and the linear term for retention time.

Results

Experiment 1

Survival to gut passage Following ingestion by Mallard, the smaller seeds of *S. lacustris* showed higher survival to gut passage (mean±SE, 125.00 ± 14.71 vs 30.83 ± 10.06, t_5= 6.92, p=0.001) and longer retention times (12.24 ± 0.46 h vs 7.78 ± 0.63, $F_{1,5}$=27.73, p=0.003) than did the larger seeds of *S. maritimus* (Fig. 1).

Percent germination Similar proportions of ingested and control seeds germinated for *S. lacustris* (41.98%±3.77 vs 48.00%±5.33, $F_{1,14}$=0.45, p=0.51) and *S. maritimus* (21.48%±8.20 vs 15.00%±5.63, $F_{1,14}$=2.88, p=0.11). However, there was a significant effect of retention time on the percentage of ingested seeds germinating. In *S. lacustris*, seeds retained for longer periods had a lower germination percentage ($F_{1,72}$=23.59, p<0.0001, Fig. 2). In contrast, in *S. maritimus*, the relationship between percent germination and retention time was quadratic, with the proportion of seeds germinating increasing at intermediate retention times and decreasing for seeds retained for more than 12 h (linear term: $F_{1,35}$=21.58, p<0.0001, quadratic term: $F_{1,35}$=14.83, p=0.0001, Fig. 2).

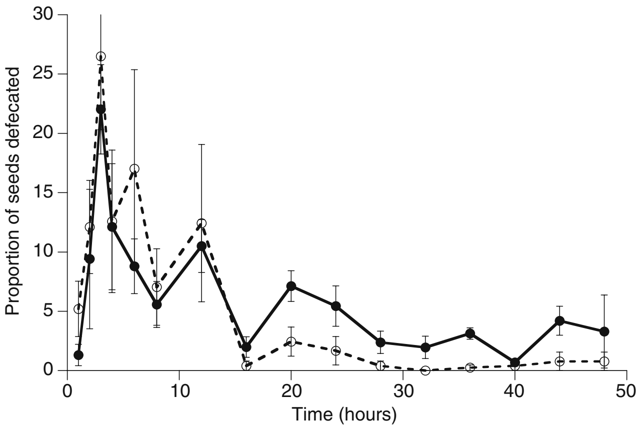

Fig. 1 Retrieval over time of the smaller *S. lacustris* (*solid line*) and larger *S. maritimus* (*dotted line*) seeds ingested by Mallard (N=6) in experiment 1, following ingestion of 300 seeds of each species at time 0

Germination rate Ingestion by ducks did not have a significant overall effect on the germination rate of *S. lacustris* seeds when compared to controls (12.94± 1.78 days vs 15.23±1.23, χ^2=2.80, 1 *df*, p=0.10). However, seeds retained for a short time period germinated

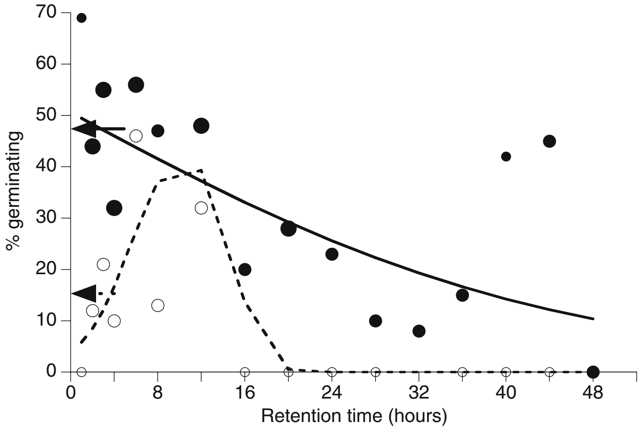

Fig. 2 Relationship between retention time and germinability of smaller *S. lacustris* (*solid line and filled circles*) and larger *S. maritimus* (*dotted line* and *open circles*) seeds ingested by Mallard (N=6) in experiment 1. *Arrows* indicate the germinability of control seeds. Number of seeds was divided into three categories of up to 10, 11 to 50 and more than 50 seeds, represented by *increasing dot sizes* in the figure

earlier than seeds retained longer (χ^2=8.20, 1 *df*, p=0.007). No evidence was found in support of a quadratic or cubic effect of retention time on germination rate for *S. lacustris* ($p \geq 0.15$). In the case of *S. maritimus*, ingested seeds germinated earlier than control seeds (46.89±5.01 days vs 78.20±6.32, χ^2=4.00, 1 *df*, p=0.005), while no linear, quadratic or cubic relationship was found between retention time and germination rate ($p \geq 0.54$).

Experiment 2

Survival to gut passage As in experiment 1, a higher number of the small-seeded species (*S. litoralis*) survived passage through waterfowl guts (74.00±11.71 vs 49.81±8.91, χ^2=9.01, 1 *df*, p=0.003). The number of seeds surviving ingestion did not differ among bird species (χ^2=0.78, 3 *df*, p=0.85, Table 1), and no interaction was detected between seed and bird species (χ^2=5.89, 3 *df*, p=0.12, Table 1).

The retention time of the smaller *S. litoralis* seeds was longer than for the larger seeds of *S. maritimus* (16.85±2.06 h vs 15.11±2.07, $F_{1,12}$=9.05, p=0.01, Fig. 3). No differences in retention time were found among bird species ($F_{3,12}$=0.60, p=0.63; seed–bird species interaction: $F_{3,12}$=1.70, p=0.22; see Fig. 3, Table 1).

Percent germination Ingestion by birds had no significant overall effect on the percentage of germinating seeds (*S. litoralis*, ingested 22.41%±6.25, control 26.00±21.69, $F_{1,24}$=0.14, p=0.71; *S. maritimus*, ingested 42.16±6.47, control 43.00±18.27, $F_{1,24}$=0.01, p=0.93). Retention time did not affect the percent germination of *S. litoralis* seeds ($F_{1,79}$=0.11, p=0.75). Bird species, however, had a significant effect on the percent germination of *S. litoralis* ($F_{3,79}$=4.21, p=0.008). A weakly significant interaction with retention time was also found ($F_{3,79}$=2.67, p=0.05). This interaction indicates that although the germination percentage of *S. litoralis* seeds defecated by Red-crested Pochard decreased significantly at longer retention times (t_{79}=2.32, p=0.02), retention time had no effect on the germinability of seeds ingested by the other three bird species ($t_{79} \leq 1.58$, $p \geq 0.12$; Fig. 4d).

Table 1 Mean±SE of the number of seeds surviving ingestion, retention time (hours) and proportion of seeds germinating after ingestion by four different duck species in experiment 2 (A=seeds of *Scirpus litoralis*, B=seeds of *S. maritimus*)

	Number of seeds surviving	Retention time (h)	Proportion germinating (%)
A			
Anas crecca	69.33±29.82	14.48±4.53	22.11±20.62
Fulica atra	83.80±23.10	18.11±3.68	27.10±13.35
Marmaronetta angustirostris	73.60±23.10	13.58±3.51	16.58±12.52
Netta rufina	63.00±29.82	23.66±4.56	29.03±17.17
B			
Anas crecca	68.33±20.96	14.11±5.15	38.32±31.14
Fulica atra	32.20±16.24	13.61±4.09	21.66±23.70
Marmaronetta angustirostris	60.00±16.24	12.22±4.01	52.51±25.39
Netta rufina	43.67±20.96	21.01±5.19	35.36±30.02

In contrast, retention time had a significant effect on the germination of *S. maritimus* seeds ($F_{1,53}$=4.01, p=0.05). This effect, however, was not linear, and a model including quadratic and cubic terms improved the fit to the data (see Fig. 4). Percent germination increased at intermediate retention times (24–48 h), but this relationship levelled off at shorter and longer periods (Fig. 4). In addition, the percentage of *S. maritimus* seeds germinating differed among bird species ($F_{3,51}$=3.85, p=0.01, Table 1), and a significant interaction between retention time and bird species was also observed ($F_{3,51}$=7.56, p=0.0003). The linear term was significant for Eurasian Teal (t_{51}=2.10, p=0.04, Fig. 4a), marginally significant for Common Coot (t_{51}=1.91, p=0.06, Fig. 4b) and not significant for the other two species ($t_{51} \leq 1.07$, $p \geq 0.29$, Fig. 4c, d). The overall result was that the percentage of seeds germinating decreased with retention time for Eurasian Teal, increased for Marbled Teal and Red-crested Pochard and showed only minor changes for Common Coot (Fig. 4).

Germination rate Ingestion by birds had no significant effects on the germination rate of *S. litoralis* seeds, as compared to controls (χ^2=2.00, 1 *df*, p=0.17). Within the bird-ingested seeds, germination rate did not differ among seeds ingested by different bird species (χ^2=3, 1 *df*, p=0.09). Retention time had a significant effect on germination rate, with seeds retained for longer periods germinating earlier (χ^2=7.20, 1 *df*, p=0.009). Such a relationship was linear, and there was no evidence in support of a quadratic or cubic effect ($p \geq 0.33$). However, when the effect of retention time was included in the model, germination rate tended to differ among bird species (marginally significant interaction: χ^2=3.6, 1 *df*, p=0.06).

In *S. maritimus*, germination rate did not differ significantly between ingested and control seeds (χ^2=0.20, 1 *df*, p=0.67). Retention time, bird species and their interaction had no effect on germination rate ($\chi^2 \leq 2$, 1 *df*, $p \geq 0.17$).

Experiment 3

There was a marginally significant trend for higher survival of small seeds of *P. pectinatus* following passage through

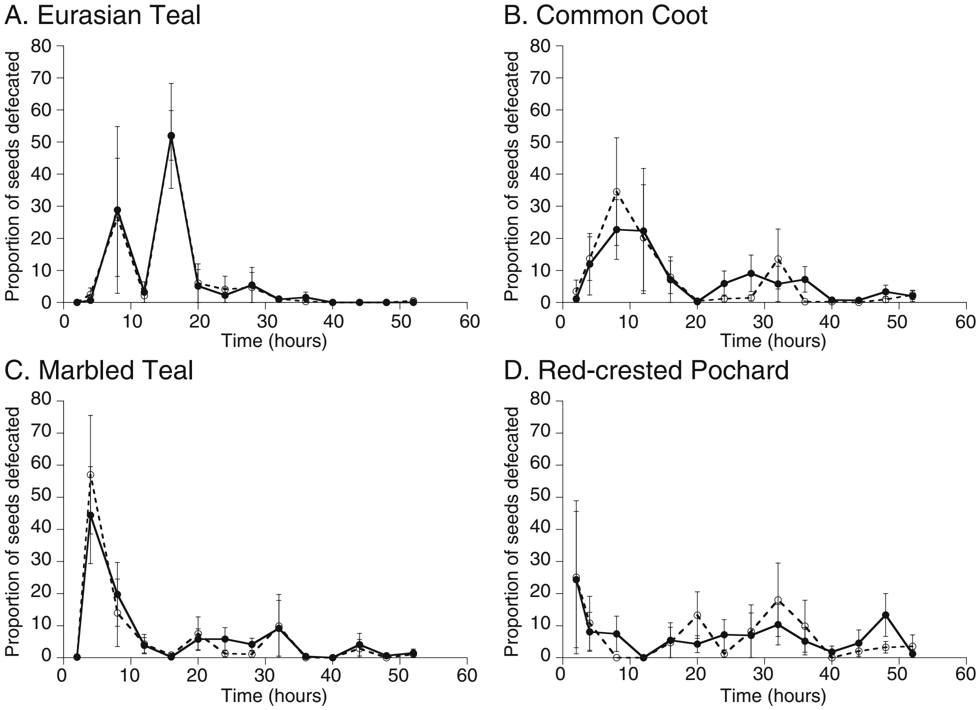

Fig. 3 Retrieval over time in experiment 2 of smaller *S. litoralis* (*solid line*, *filled circles*) and larger *S. maritimus* (*dashed line*, *open circles*) seeds ingested by **a** Eurasian Teal (N=3), **b** Common Coot (N=5), **c** Marbled Teal (N=5) and **d** Red-crested Pochard (N=3)

Marbled Teal guts (t_9=2.15, p=0.06). Only a fairly low number of small seeds (1.70±0.79 seeds per bird) survived gut passage. When testing the germinability of control (non-ingested) seeds, a higher proportion of larger-sized seeds germinated (32.36±7.60 vs 16.00±7.11, $F_{1,65}$=6.15, p=0.02). The percent germination of large and small seeds after ingestion could not be compared since only small seeds survived ingestion.

Discussion

The importance of seed size for endozoochorous dispersal

The effects of seed ingestion by vertebrates on germination patterns have received considerable attention (reviewed in Traveset and Verdú 2002). However, the potential relationship between seed size and resistance to gut passage has

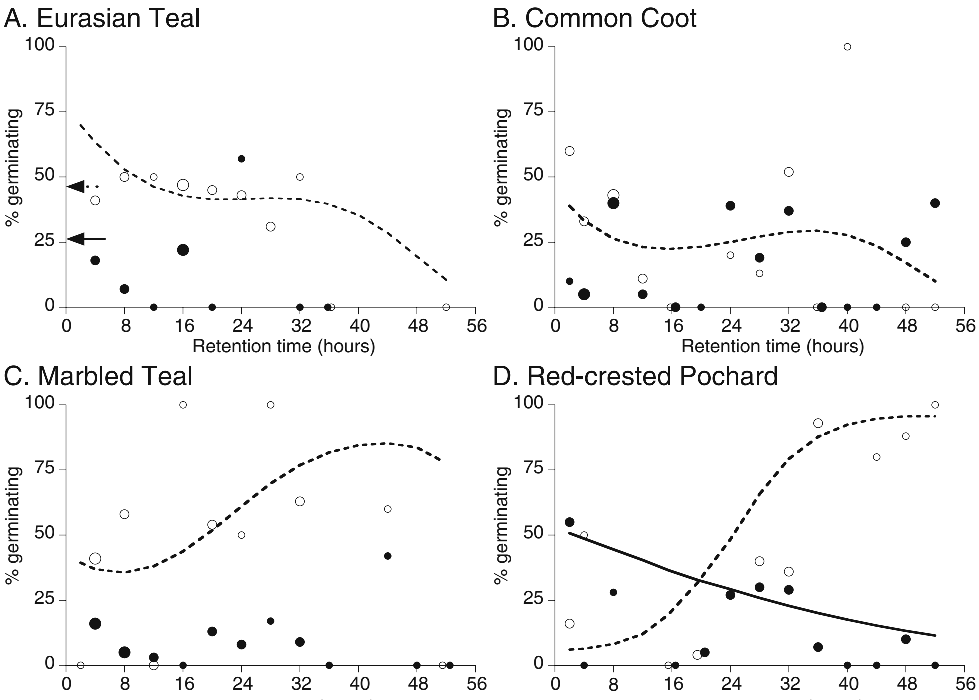

Fig. 4 Relationship between retention time and germinability of smaller *S. litoralis* (*solid line*, *filled circles*) and larger *S. maritimus* (*dashed line*, *open circles*) in experiment 2 for **a** Eurasian Teal (N=3), **b** Common Coot (N=5), **c** Marbled Teal (N=5) and **d** Red-crested Pochard (N=3). Only statistically significant regression lines are shown. *Arrows* indicate mean germinability of control seeds. Number of seeds was divided into three categories of up to 10, 11 to 50 and more than 50 seeds, represented by *increasing dot sizes* in the figure

been examined in only a few studies. De Vlaming and Proctor (1968) examined the resistance of seeds of 23 species of predominantly aquatic plants to ingestion by Mallard and Killdeer (*Charadrius vociferous*) and concluded that species with smaller seeds were more resistant to ingestion, although no statistical analyses were presented. Similarly, Holt and van der Valk (2002) found a negative relationship between seed size and resistance of seeds to gut passage for wetland plant species, although again no statistical analyses were performed. Analysis of data in their Table 2 suggests that the correlation was not significant (Spearman's rho=−0.48, p=0.19). In more recent studies relying on statistical analyses, Wongsriphuek et al. (2008) concluded that size was unrelated to resistance to digestion by birds, while Soons et al. (2008) concluded that small seeds survived better. Hence, the relationship between seed size and resistance to gut passage requires further investigation. Although strong inference cannot be derived when comparing only two species (see Garland and Adolph 1994), we found a higher resistance to gut passage and longer retention times for smaller seeds when comparing three *Scirpus* species, as well as small and large seeds of *P. pectinatus*, all of which are widely consumed by waterfowl (Green et al. 2002; Brochet et al. 2009).

Our results indicate that smaller seed size facilitates internal transport through two complementary mechanisms. Firstly, a larger proportion of small seeds survived ingestion. Secondly, small seeds were retained for a longer time inside the gut and thus could be dispersed over longer distances compared to larger seeds. Furthermore, we found evidence that these factors are relevant both at the intra- and inter-specific levels. The relationship between seed size and retention time has been documented in terrestrial plants (Barnea et al. 1991, see Traveset 1998 for review). However, the benefits of small size for dispersal are reduced, at least at the intra-specific level, by the lower germinability and generally lower competitive ability of small seeds, shown here for *P. pectinatus* and previously reported for other species (Fenner 1985; Winn 1985; Wulff 1986). Our results indicate a trade-off between dispersal and competitive ability, mediated by variation in seed size, in agreement with other studies (Smith and Fretwell 1974; Westoby et al. 1992, 1996; Geritz 1995; Jakobson and Eriksson 2000). Alternatively, small *P. pectinatus* seeds could have included a larger proportion of immature and/or undeveloped seeds, which would explain their reduced percentage germination. In addition to size, seeds used in our experiment may have differed in other variables that affect the capacity to survive gut passage (e.g. coat thickness), although it seems unlikely that small seeds would have thicker coats than large seeds (see also discussion in Traveset et al. 2001).

Differences in seed survival and germination among bird species

Similarly to Charalambidou et al. (2003), we did not detect any overall effect of the disperser species on the proportion of seeds surviving gut passage. In contrast, a field study involving these and other waterbird species reported differences among bird species in relation to the proportion of *R. maritima* seeds destroyed during digestion (Figuerola et al. 2002). The lack of inter-specific differences in the experimental trials could be related to the homogeneity of the birds' diet. In the field, different waterbird species often have different diets (Green et al. 2002), which may result in short- and long-term differences in seed survival after gut passage, mediated by changes in food retention time and/or by changes in gut structure and physiology resulting from diet acclimatisation (Kehoe and Ankney 1985; Liukkonen-Anttila et al. 2000). Long-term acclimation to animal- and seed-based diets in captive mallard resulted in significant differences in the survival of *P. pectinatus* seeds following gut passage, although seed retention time was unaffected (Charalambidou et al. 2005).

For the plant species that were tested, we found no overall differences in percent germination among ingested and non-ingested seeds. Although we cannot be sure that some of the seeds that did not germinate were in dormancy, all seeds were exposed to two germination trials separated by cold stratification, aiming to break dormancy (Clevering 1995; Santamaría et al. 2002). In contrast, the effect of retention time was significant in most cases. Hence, ingestion by birds affected germination of seeds according to the time they were retained inside the gut, but no overall difference with control seeds was detectable when germination rates were averaged for all seeds irrespective of their retention times. In most cases, the percentage of seeds germinating decreased in seeds retained longer than 40 h, thus reducing the capacity for long distance dispersal (Figs. 2 and 4), although viable seeds were still being recovered at 52 h after ingestion. In addition, the effect of retention time on percent germination differed among seed and bird species. In the case of *S. litoralis*, the reduced germination percentage of seeds ingested by Red-crested Pochard was most likely the result of the harsher treatment in the gut of this large, predominantly herbivorous species, since birds with more fibrous diets have heavier gizzards and longer intestines (Barnes and Thomas 1987; Piersma et al. 1993).

The impact of retention time on the viability of seeds

The disparity observed among bird species in the effects of retention time on the percentage of seeds germinating is consistent with the diversity of effects reported for ingestion by birds and other vertebrates, on germination patterns of seeds (Traveset and Verdú 2002). Our results indicate that differences in retention time may help explain the variation of effects reported in different studies. However, they also highlight a methodological problem when comparing effects of ingestion by vertebrates: The conclusions reached in these studies may depend on retention time. Clearly, retention time merits further attention in future studies dealing with the effects of ingestion by vertebrates on germination patterns, especially when working with species with long, or highly variable, retention times. Previous studies of the effects of retention time on germination compared the effects of ingestion by bird species with different retention times (e.g. Barnea et al. 1991) or failed to control for related effects of bird individuals (Soons et al. 2008; Wongsriphuek et al. 2008). Individual variation within a bird species is an important factor due to high interindividual variation in gut structure and function that affect seed passage and posterior germination (Charalambidou et al. 2003).

Both Soons et al. (2008) and Wongsriphuek et al. (2008) detected a negative effect of retention time on seed viability but failed to control for the effects related to bird individuals. To our knowledge, only Murray et al. (1994) and Charalambidou et al. (2003) studied the effect of seeds retained in the gut over different times by the same individuals. Murray et al. (1994) provided fruits of *Witheringia solanacea* (Solanaceae) to captive Black-faced Solitaires *Myadestes melanops* and showed that seeds defecated rapidly after ingestion were more likely to germinate than those retained for longer inside the guts. In this system, retention times were short (up to 60 min), probably because of the small size of the birds and the laxative effect of the pulp (Murray et al. 1994). Charalambidou et al. (2003) and Pollux et al. (2005) reported reductions in germinability of, respectively, *R.*

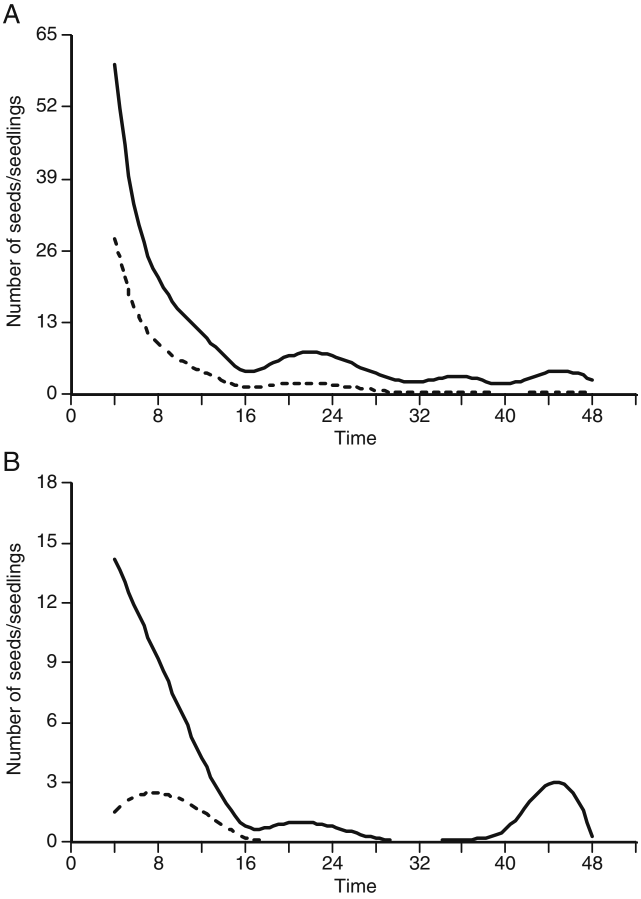

Fig. 5 Expected modification of effective seed shadows by the effects of retention time on germinability as estimated from the results of experiment 1. Number of seeds defecated in each period of 4 h (*solid line*) and expected number of seeds germinating (*dotted line*) for smaller *S. lacustris* (**a**) and larger *S. maritimus* (**b**) seeds. The difference between the two variables was most pronounced for *S. maritimus* for which, at long retention times, the negative impact on viability greatly reduces the expected number of seedlings

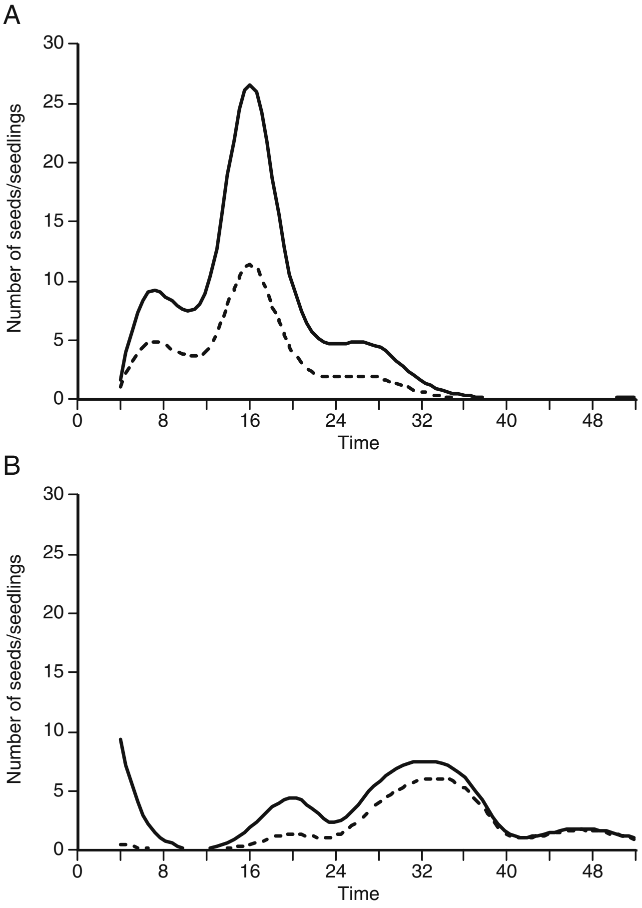

Fig. 6 Expected differences in the modification of *S. maritimus* effective seed shadows produced by **a** Eurasian Teal and **b** Red-crested Pochard in experiment 2. The number of seeds defecated in each 4-h interval is shown by the *solid line* and the expected number of seedlings generated from these seeds by the *dotted line*

maritima and *Sparganium emersum* seeds retained for longer times inside duck guts. In general, all these papers compare seed with largely different morphologies, while our study chose to compare seeds of comparable morphology that differed mainly in size. We have expanded these results by analysing the effects of retention time on germination patterns of seeds of several non-fleshy fruiting species, showing that retention time has an effect on germination patterns that is independent of differences in the structure of the gut at the inter- or intra-specific level. Our results also indicate that the effects of retention time on germination patterns are non-linear and that percent germination, in some cases, increases for seeds retained for intermediate periods.

We also detected contrasting effects of retention time on germination rates of different plant species: While longer retention times increased the germination rate in *S. litoralis*, they had the opposite effect on *S. lacustris* and no effect in *S. maritimus*. Ours is the first study to analyse the possible effects of retention time on germination rate, and we cannot provide a clear explanation for this disparity of effects, other than those usually cited to justify the variation of effects of ingestion by vertebrates on germination patterns: differences in seed sculpture, seed age or secondary effects derived from natural levels of seed dormancy (Traveset 1998).

The consequences for seedling distribution

Retention time has been demonstrated to determine the size of seed shadows produced by frugivorous birds (Holbrook and Smith 2000), with larger seed shadows for plants with small seeds and produced by birds with longer retention times. However, long retention time is not the only factor necessary for long distance dispersal, given that seeds should remain viable after transport. Our results demonstrate that retention time not only affects dispersal distance but can also secondarily modify seedling distribution through the effects of retention time on seed germination and the probability of seedling establishment (see Fig. 5). However, our results also demonstrate that the relationship between percent germination and retention time is a highly variable function of both bird and plant species. Thus, it is not yet possible to generalise about how seed shadows will be modified by differences in percent germination of seeds as a function of retention time (Fig. 6). Furthermore, the shape of the relation between retention time and dispersal distance will depend on the ecology of the disperser species (Higgins et al. 2003). In the case of Anatidae, the area used in a 24-h interval can range from just a few hundred metres in individuals feeding and resting in the same wetland, to several tens of kilometres when ducks are feeding on grasslands or fields close to roost sites (Guillemain et al. 2002), or to more than 1,200 km during migration (Clausen et al. 2002). Thus, more studies are needed that quantify both animal movements and seed dispersal curves (Higgins et al. 2003).

In conclusion, retention time has important effects on the percentage of seeds germinating and germination rate, which in turn have a significant bearing on dispersal curves. These are likely to be reflected in the resulting effective dispersal shadows. These effects are however complex and depend on the seed species and bird vectors involved, ranging (in the time range studied by us) from linear reductions to non-linear increases in the germinability of seeds and also including optima at intermediate time periods.

Acknowledgments Doñana National Park allowed us to use their installations. We are grateful to Pablo Pereira, Begoña Gutíerrez, Cristina Belén Sánchez and Celia Sánchez for their help during the setup of the experiments. Pedro Jordano and two anonymous referees provided valuable comments on an earlier version of the manuscript. Our research was supported by the European Union project "LAKES—Long distance dispersal of Aquatic Key Species" (ENV4-CT97-0585), by the Spanish Ministry of Science and Technology (project BOS2000-1368), and ESF project BIOPOOL (project CGL2006-27125-E/BOS from the Spanish Ministry of Education and Science).

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