



Endozoochory of Mediterranean aquatic plant seeds by teal after a period of desiccation: Determinants of seed survival and influence of retention time on germinability and viability

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ABSTRACT

The capacity of eight wetland seeds to be internally dispersed by common teal (*Anas crecca* L.) after a dry period typical of natural Mediterranean wetlands was experimentally quantified. These plant taxa (*Chara* spp., *Echinochloa crus-galli* (L.) P. Beauv., *Eleocharis palustris* (L.) Roem. & Schult., *Polygonum lapathifolium* L., *Potamogeton nodosus* Poir., *Potamogeton pusillus* L., *Schoenoplectus mucronatus* (L.) Palla, *Scirpus maritimus* L.) were frequently ingested by teal in the field. Captive teal were fed with known quantities of dry seeds and faecal samples were collected at intervals of 1–2 h for 48 h. The proportion of seeds retrieved after gut passage that germinated varied between taxa from 3% to 83%. From 2% to 63% of the seeds ingested were recovered intact, and 18–95% of these were still viable. Therefore, an overall 32% of the seeds ingested were evacuated in a viable condition, ranging from 0.2% for *Chara* spp. to 54% for *S. mucronatus*. Mean seed retention time was 3–5 h. Overall, 94% of seeds were evacuated within 12 h, but 2% were recovered after more than 24 h. Seed viability was reduced at longer retention times, although viable seeds of all taxa except *Chara* spp. were recovered 44–48 h after the experiment began. Germinability was increased by gut passage, although for some species this effect was only significant after stratification, indicating that they exhibit both mechanical and physical dormancy. Seed germination was accelerated by gut passage, especially at longer retention times. Gut passage increased the proportion of viable seeds, suggesting selective digestion of non-viable ones. Endozoochorous transport by teal appears to be a widespread dispersal mechanism among wetland plants.

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1. Introduction

Aquatic plants inhabit naturally fragmented habitats, but often occupy wide geographic ranges (Santamaría, 2002; Chambers et al., 2008), suggesting that they have efficient vectors of passive seed dispersal. Waterfowl (ducks, geese, swans) have long been suggested to play a role in the dispersal of aquatic seeds (Darwin, 1859; Ridley, 1930). These birds are abundant and widely distributed, ingest aquatic seeds and regularly undertake movements

between river catchments and isolated waterbodies (Green et al., 2002).

The potential importance of seed dispersal via passage through waterfowl guts (endozoochory) was recognized a long time ago (e.g. Ridley, 1930). Since then, field studies have shown that droppings of ducks can contain a variety of viable seeds (Holt Mueller and van der Valk, 2002; Figuerola et al., 2003; Charalambidou and Santamaría, 2005; Green et al., 2008). Moreover, experimental studies have investigated the potential for endozoochory by waterfowl (reviewed in Charalambidou and Santamaría, 2002; Figuerola and Green, 2002). However, only recent studies have conducted controlled experiments to provide a quantitative comparison of waterfowl-mediated dispersal potential among wetland plants, and compared how seed properties (e.g. size and fibre content) influence seed retrieval, retention and germination (e.g. Holt Mueller and van der Valk, 2002; Soons et al., 2008; Wongsriphuek et al., 2008). These studies have relied heavily on a single, relatively sedentary duck species (the mallard *Anas platyrhynchos* L.)

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and on plant species occurring in temperate regions. Consequently, more comparative studies on a wide range of plant species using other migratory duck species are needed to identify seed characteristics determining the probability of dispersal by waterfowl and the potential for long-distance dispersal.

To study the potential contribution of waterfowl to wetland plant dispersal in the Mediterranean region, we fed common teal (*A. crecca*), a granivorous long-distance migrant, with seeds of eight common plant taxa, representing a wide range of seed traits (size, mass and hardness). Field data (Brochet et al., 2009, 2010) confirm that teal ingest these seed taxa as they winter in the Camargue (southern France) (relative frequencies of occurrence in oesophagi and gizzards ranging from 19 to 92% and average relative dry weights ranging from 1 to 32%, $n = 302$ teal, Brochet et al., unpublished data). The Camargue covers 140,000 ha in the Rhone Delta, and hundreds of thousands of ducks spend the winter there or stop during migration. Annual peak counts of teal average 36,000 birds (Tamisier and Dehorter, 1999). For the eight seed taxa, survival after gut passage, retention time and subsequent germinability and viability were quantified. These are processes that partly determine dispersal probabilities. This was done after simulating dry periods that are regularly experienced by seed banks in natural Mediterranean wetlands which reflood in winter (Espinari et al., 2005). Specifically, the following interrelated predictions were tested:

- (i) Seed survival through the digestive tract is dependent on seed traits: species with smaller and/or harder seeds should resist digestion more effectively and thus have higher retrieval, germinability and viability. Small size should reduce retention time in the digestive system.
- (ii) Seed taxa with a shorter retention time should have higher retrieval, germinability and viability.
- (iii) For a given taxon, seeds with a longer retention time should have reduced germinability and viability. However, germination rate should increase with longer retention times, as mechanical dormancy is more likely to be broken.
- (iv) For early-maturing plants, seeds passing through the gut should germinate more often and faster than control seeds, because gut passage breaks mechanical seed dormancy. However, for late-maturing plants that have physical dormancy, these effects of gut passage should only be discernable following winter stratification.

2. Methods

2.1. Seed collection and measurement

The term 'seed' is used hereafter to refer to seeds plus oospores produced by *Chara* algae. Seeds of eight plant taxa (*Chara* spp., *Echinochloa crus-galli*, *Eleocharis palustris*, *Polygonum lapathifolium*, *Potamogeton nodosus*, *Potamogeton pusillus*, *Schoenoplectus mucronatus*, *Scirpus maritimus*) were collected in the field in summer 2007 and were cleaned, dried and stored in glass vials at room temperature and natural light schedule in the lab until the experiment the following spring.

Dried seed hardness was measured using a small manual device, designed to measure soil hardness (Lepont équipement, Chasse sur Rhône, France), which applied increasing pressure from 0.75 to 10.00 kg.cm⁻², at intervals of 0.25 kg.cm⁻². Dried seed maximum length (hereafter 'length') was measured on graph paper under a binocular microscope, to the nearest 0.02 mm. For both parameters, 10 seeds per species (from the same batches of dried seeds used in the experiments) were measured. Seed mass was calculated by weighing together 30 seeds per species, oven-dried beforehand at 60 °C during 24 h.

2.2. Feeding experiment

Feeding trials were conducted from 25 March through 9 May 2008 at the Tour du Valat, France, using 12 captive teal, seven males and five females (live body weight: 267.4 ± 9.4 g [$x \pm SE$]), born in captivity (seven in July 2005 and five in July 2007). Prior to the trials, teal were housed outdoors and fed since hatching on a stable diet of commercial pellets (Stargib-Entretien, Longue Jumelles, France) with free access to grit and water. Feeding trials were approved by the "Direction Départementale des Services Vétérinaires".

Previous comparative studies of seed digestion by ducks generally tested one seed species at a time (e.g. Holt Mueller and van der Valk, 2002; Soons et al., 2008; Wongsriphuek et al., 2008). However, ducks have a mixed diet in nature (Cramp and Simmons, 1977), so we chose to feed different seed taxa at the same time to a given individual. At the beginning of each replicate (six in total per individual), each teal was force-fed 800 seeds (100 seeds per taxa) by inserting a tube (0.9 cm × 8 cm) into the oesophagus and carefully putting the seeds into the tube using a funnel (Dugger et al., 2007). Seeds that were regurgitated (eight seeds on average per bird, ranging from an average of one for *Chara* sp. to 14 for *S. maritimus*) or stuck inside the tube (three seeds on average per bird, ranging from one for *P. nodosus* to eight for *Chara*) were counted, to correct the exact number of seeds ingested. Each teal was then kept for 48 h in an individual cage (60 cm × 50 cm × 50 cm) with a mesh floor (9 mm) and removable plastic trays under each cage. Food pellets, water and grit were available *ad libitum*. The birds were released into the outdoor pen 48 h after ingestion, until the next replicate five days later.

Teal faeces were collected in removable trays every hour for the first four hours and then every two hours up to 48 h after seed ingestion. The bowls containing food, grit and water were also checked to recover any faeces. Faeces were immediately sieved (63 µm mesh) in the lab. Intact seeds were then retrieved and counted.

2.3. Germination and viability tests

160 seeds (20 per taxa) per replicate were used as controls for germination tests. Retrieved and control seeds were set to germinate in microtitre trays filled with bottled mineral water (Volvic, France; conductivity: 170–200 µS cm⁻¹) with up to 30 seeds per cell for the smallest and 15 for the largest taxa. The *Chara* controls and oospores retrieved from a given individual at each collection time were germinated in a small Pétri dish, because they tended to develop fungi in microtitre trays. Trays were positioned in the lab at room temperature and light conditions. Except for *P. nodosus*, these germination conditions had already been showed to fit the requirement of these taxa (Brochet et al., 2010). Seed germination was checked every 2–3 days during 56 days. At each check, water was replenished and germinated seeds were counted and removed from the trays. The few seeds that became infected by bacteria and/or fungi were removed to avoid contamination of remaining seeds (and were excluded from all analyses). After 56 days, non-germinated seeds were removed from the lab and stored in mineral water at 4 °C in the dark in the fridge (simulating winter stratification). After 12 weeks of stratification, seeds were set for a second germination trial (same conditions). Mean temperature of the lab was 22.3 °C, ranging from 16 to 32 °C over the whole germination test period. There was no significant difference in mean temperature between germination trials (t -test: $t = 0.793$, $p = 0.431$). Photoperiod was longer during the first germination test (during spring and summer: 14.4 ± 0.4 h [$x \pm SE$]) than during the second one (during autumn: 11.1 ± 0.9 h) (t -test: $t = 3.333$, $p = 0.028$).

Ungerminated seeds were tested for viability using a standard tetrazolium method (De Vlaming and Proctor, 1968). Seeds were

cut to expose embryos and placed on a filter paper saturated with 1% tetrazolium solution in a Pétéri dish, for 24 h in the dark at room temperature. Seeds with a positive tetrazolium response (i.e. embryos were respiring and turning red) were assumed to be viable (Nachlas et al., 1960).

2.4. Statistical analysis

The effects of teal age, sex and their interaction on the total proportion of retrieved, germinated or viable seeds were tested using generalized linear mixed models (GLMMs) with a binomial error distribution and a log link function. Hereafter, viability refers to seeds that germinated or showed a positive tetrazolium response. Teal and replicates were included as random factors (replicates were nested within teal). The total number of ingested seeds (for seed retrieval) and the total number of seeds entering the germination trials (for seed germinability and viability) were also included in the model as a binomial denominator. Models of age and sex did not differ significantly from null models, with the following exception: the model of the proportion of germinated seeds that included only age (estimate = -0.076 ± 0.032 , $p = 0.018$, with more germination for teal hatched in 2005) was significantly different from the null model ($\chi^2 = 4.296$, $p = 0.038$). Thus, so as to focus on general patterns more relevant to nature, teal sex and age were not considered in further analyses.

2.4.1. Effect of seed characteristics

The means of seed length, mass and hardness were highly inter-correlated between taxa (Pearson correlations: all r -values > 0.736 , all p -values < 0.023). Consequently, only seed length was used in further analyses. Substitution with hardness or mass made no difference (results not shown). Seed length was significantly different between taxa (ANOVA: $F = 518.76$, $p < 0.001$) and *P. lapathifolium*–*S. mucronatus* was the only pair that did not significantly differ (Tukey test: $p = 0.600$).

The effect of seed length (SL) on the total proportion of retrieved, germinated or viable seeds was tested using linear models. For each dependent variable, data were pooled per taxon ($n = 8$). Dependent variables were normally distributed after arcsine transformation. A quadratic factor for seed length (SL²) was also tested in the models (since the effect of SL is not necessarily linear). Null and initial models were compared via analyses of variance.

2.4.2. Effects of gut passage and retention time on seed germinability, viability and germination time

The effect of treatment (i.e. ingested versus control seeds, Ttt) on the proportion of germinated or viable seeds was tested using GLMMs with a binomial error distribution and a log link function. Seed taxon (ST, a factor of eight levels) was also tested in the initial models, as well as its interaction with treatment. Similar random factors and binomial denominators to those described above were also included in the models. We then tested the treatment effect on the mean proportion of viable seeds that germinated after the first and second germination trials for each taxon with paired t -tests (comparing control vs. ingested seeds for each replicate).

Effects of retention time (RT) were analyzed with similar GLMMs to those for treatment, but excluding the control seeds from the dataset. The quadratic factor of retention time (i.e. RT²) was also tested in the initial models.

Effects of Ttt and RT on seed germination time (the inverse of germination rate) were also tested using similar GLMMs with a Poisson error distribution. “Germination time” was the number of days between setting for germination and the germination event (i.e. when a visible root tip was first observed) for each seed. Only data from seeds that germinated by the end of both germination trials were included, to separate the effects of gut passage on ger-

Table 1

Mean seed traits (\pm SE) of eight seed taxa ingested by teal. Measurements were made on dried seeds.

Seed taxon	Length (mm)	Mass (mg)	Hardness (kg cm ⁻²)
<i>Chara</i> spp. ^a	0.60 \pm 0.05	0.04	0.75 \pm 0.00
<i>Echinochloa crus-galli</i>	2.66 \pm 0.05	2.21	2.28 \pm 0.30
<i>Eleocharis palustris</i>	1.43 \pm 0.06	0.65	2.13 \pm 0.09
<i>Polygonum lapathifolium</i>	2.27 \pm 0.03	2.07	6.73 \pm 0.74
<i>Potamogeton nodosus</i>	3.55 \pm 0.05	4.43	6.70 \pm 0.20
<i>Potamogeton pusillus</i>	1.88 \pm 0.03	0.67	2.78 \pm 0.22
<i>Schoenoplectus mucronatus</i>	2.17 \pm 0.03	1.17	3.23 \pm 0.09
<i>Scirpus maritimus</i>	3.01 \pm 0.04	2.25	7.38 \pm 0.54

^a Likely to include *C. vulgaris*, *C. globularis* and *C. aspera*, the dominant *Chara* species in the study area (J.B. Mouronval pers. commun.).

mination time from the effects on germinability. Germination trials (TR a fixed factor) and the interactions RT*ST and ST*TR were added to initial models.

For each GLMM analysis, all possible subsets of the full model, i.e. all possible combinations of independent variables and interactions, were tested. Then, the Akaike Information Criterion (AIC) was used to compare different models. The best one had the smallest AIC value. However, if the difference of AIC (Δ AIC) between two models was less than two, they were considered to be equivalent (Mc Cullagh and Nelder, 1989). When several ‘best models’ were found, a model-averaging approach (Burnham and Anderson, 2002) was used to obtain final estimates and standard errors of the parameters associated with each variable. Over-dispersion was later controlled for, but significant effects were unchanged (results not shown).

R software (version 2.8.1) was used for all statistical analyses (R Development Core Team, 2008).

3. Results

Viable seeds of all plant taxa were retrieved from teal faeces. In total 20,550 intact seeds were collected, of which 16,182 were viable and 11,339 germinated (Fig. 1). Mean seed length varied from 0.6 mm for *Chara* spp. to 3.6 mm for *P. nodosus* (Table 1). For all seed taxa, median and mode retention time were 2 or 3 h (Table 2). Overall, 94% of retrieved and viable seeds were evacuated within 12 h, but a total of 340 retrieved seeds (2%) and 255 viable seeds (1.6%) were recovered after more than 24 h. Two groups of taxa could be distinguished: (1) *Chara* and *E. crus-galli* had low survival (retrieval of $< 13\%$) and short retention times (mean < 3.3 h for viable seeds); and (2) the other taxa had higher but variable retrievals (30–65%), and almost identical mean retention times (Table 2). For these six taxa, retrieval was thus independent of retention time. Percentage seed retrieval ranged from 2% for *Chara* spp. to 63% for *E. palustris* (Table 2).

3.1. Effects of seed characteristics

No effect of seed length was found on the total proportion of retrieved, germinated or viable seeds. Null and initial models did not significantly differ (all F -values > 0.030 , all p -values > 0.347).

3.2. Effects of gut passage and retention time on seed germinability, viability and germination time

One single best model was found for the effects of gut passage on germinability and viability (Table S1). Gut passage generally increased the proportions of germinated and viable seeds (Table 2 and 3a). Strong Ttt*ST interactions showed that the effects of gut passage on both germinability and viability were taxa specific (Table 3a). After the first germination trial, the proportion of

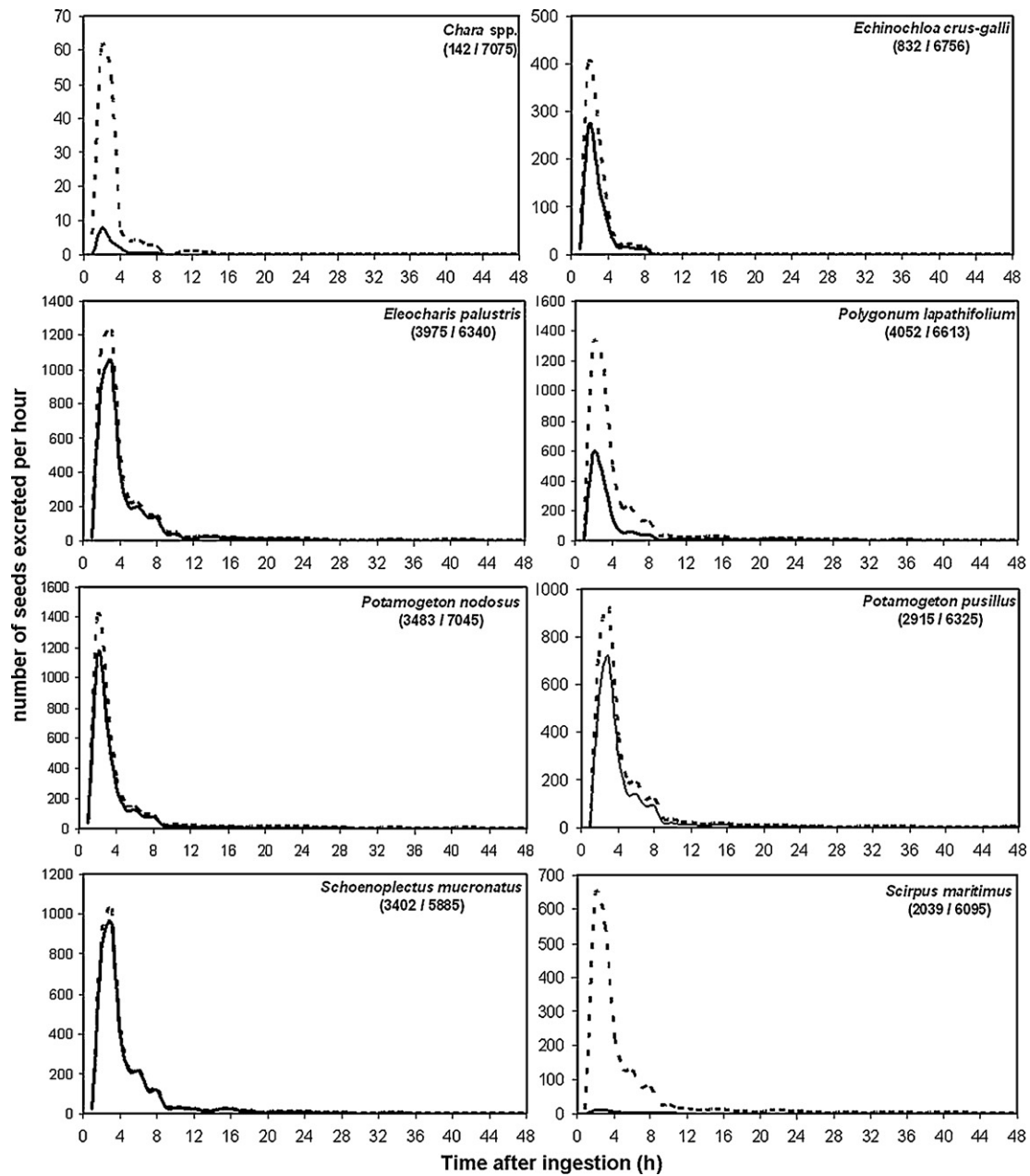


Fig. 1. Mean numbers of retrieved (dashed line) and viable (solid line) seeds recovered intact in teal faeces according to retention time (numbers in brackets: number of seeds retrieved/total number of seeds ingested). Viability refers to seeds that germinated or showed a positive tetrazolium response.

Table 2
Mean (\pm SE), mode, median and maximum retention time (in h) for retrieved (R) and viable (V) seeds, and percentages of retrieved, germinated and viable seeds for teal-ingested (I) and control (C) seeds. Mode and median were equal for retrieved and viable seeds. Germinability is the percentage of intact seeds that germinated. Viability is the percentage of intact seeds that germinated or showed a positive tetrazolium response. The experiment was ended at 48 h.

Seed taxon	Retention time				Retrieval (%)		Germinability (%)		Viability (%)		
	Mean		Mode	Median	Maximum	I	C	I	C		
	R	V									
<i>Chara</i> spp.	5.0 \pm 0.32	3.1 \pm 0.78	3	3	14	8	2 \pm 0.2	12 \pm 3	0 \pm 0	18 \pm 4	9 \pm 3
<i>Echinochloa crus-galli</i>	3.2 \pm 0.18	3.2 \pm 0.22	2	2	44	44	12 \pm 0.4	53 \pm 2	36 \pm 4	68 \pm 2	74 \pm 4
<i>Eleocharis palustris</i>	5.1 \pm 0.19	4.9 \pm 0.21	3	3	48	48	63 \pm 1	50 \pm 1	11 \pm 3	85 \pm 1	74 \pm 4
<i>Polygonum lapathifolium</i>	4.8 \pm 0.19	4.8 \pm 0.20	2	3	48	48	61 \pm 1	38 \pm 1	18 \pm 4	88 \pm 1	85 \pm 3
<i>Potamogeton nodosus</i>	4.8 \pm 0.21	4.8 \pm 0.24	2	3	48	48	49 \pm 1	77 \pm 1	50 \pm 5	83 \pm 1	65 \pm 4
<i>Potamogeton pusillus</i>	5.2 \pm 0.21	4.9 \pm 0.25	3	3	48	48	46 \pm 1	70 \pm 1	48 \pm 5	78 \pm 1	70 \pm 4
<i>Schoenoplectus mucronatus</i>	5.1 \pm 0.19	5.1 \pm 0.21	3	3	48	48	58 \pm 1	83 \pm 1	56 \pm 5	95 \pm 0.4	85 \pm 3
<i>Scirpus maritimus</i>	5.0 \pm 0.22	5.3 \pm 0.48	2	3	48	44	34 \pm 1	3 \pm 0.4	2 \pm 1	22 \pm 1	19 \pm 4

Table 3

Results of model averaging based on alternative GLMMs (see Table S1) presenting final estimate (β), standard-error (SE) and p -value (* <0.05 , ** <0.01 , *** <0.001 , ns: non-significant) for proportion of germinated and viable seeds and germination time, for (a) treatment (Tt, i.e. ingested versus control seeds), and (b) retention time (RT) analyses. Seed taxon (ST) and germination trial (TR) were also included in the initial models. Viability refers to seeds that germinated or showed a positive tetrazolium response.

Variables	Germinated			Viable			Germination time		
	β	SE	p	β	SE	p	β	SE	p
a.									
Ttt ^a	1.871	0.185	***	1.866	0.178	***	-0.238	0.88	**
ST	0.135	0.033	***	0.050	0.030	ns	0.155	0.009	***
Ttt*ST	-0.151	0.034	***	-0.219	0.031	***	-0.006	0.007	ns
TR							-0.951	0.012	***
ST*TR							-0.060	0.002	***
b.									
RT	-0.180	0.019	***	-0.214	0.017	***	-0.315	0.054	***
RT ²	0.003	0.0004	***	0.003	0.0004	***	0.006	0.002	**
ST	0.046	0.012	***	-0.032	0.011	**	0.881	0.072	***
RT*ST	-0.005	0.0004	*	-0.002	0.002	ns	-	-	-
TR ^b							-8.002	0.582	***
ST*TR							-2.505	0.113	***

^a Ingested seeds had higher germinability and viability and a lower germination time

^b Germination time was lower in the second trial.

viable seeds that germinated was significantly higher after ingestion than for control seeds only for *E. palustris*, *P. nodosus* and *S. maritimus* (paired t -tests: all t -values > 3.088 , all p -values < 0.027). After the second germination trial, significant positive effects of gut passage on germinability were observed for *E. crus-galli*, *E.*

palustris and *P. lapathifolium* (paired t -tests: all t -values > 2.867 , all p -values < 0.018). Both for control and ingested seeds, most *P. nodosus* and *P. pusillus* germinated after stratification (proportion of viable seeds that germinated as follows: for *P. nodosus*: 1st trial 0.02 ± 0.01 for control, 0.37 ± 0.05 for ingested; 2nd trial

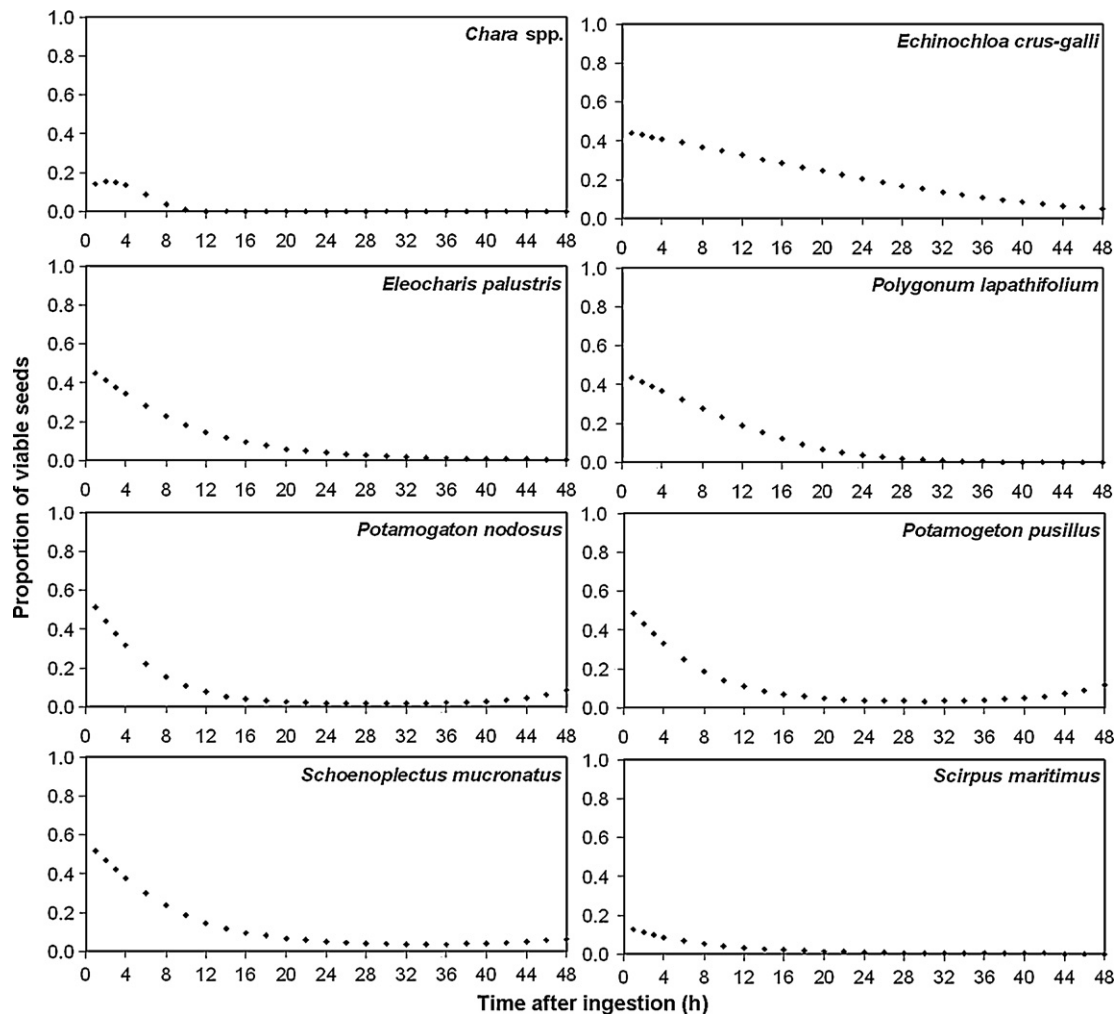


Fig. 2. Proportion of viable seeds according to retention time. Logistic equations were fitted according to the results of GLMMs of the effect of retention time for each species (see Section 2.4.2 and Table 3b). Viability refers to seeds that germinated or showed a positive tetrazolium response.

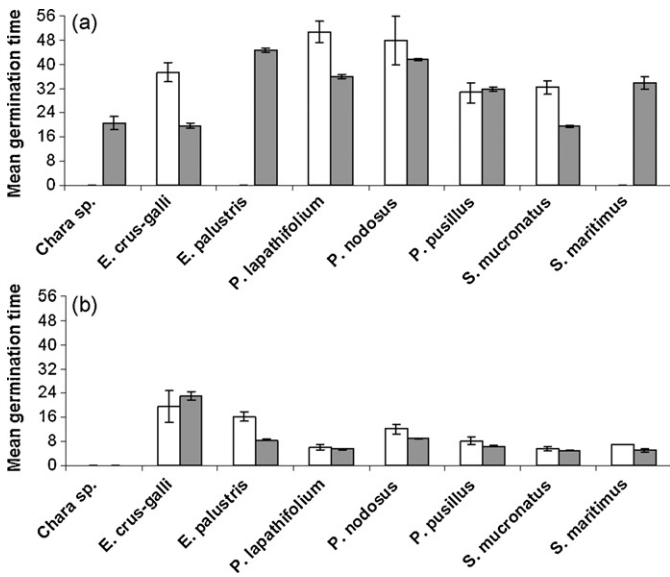


Fig. 3. Mean germination time of germinated seeds after (a) the first and (b) the second germination trial for control (white) and ingested (grey) seeds. Error bars indicate \pm standard error for six replicates.

0.77 ± 0.09 for control, 0.89 ± 0.02 for ingested. For *P. pusillus*: 1st trial 0.02 ± 0.08 for control, 0.26 ± 0.06 for ingested; 2nd trial 0.57 ± 0.13 for control, 0.80 ± 0.04 for ingested).

One single best model was found for the effect of retention time (RT) on seed germinability and two for its effect on seed viability (Table S1). RT had a curvilinear effect on both variables but, within the range studied, germinability and viability decreased consistently with retention time (Table 3b, Fig. 2). An RT*ST interaction was significant for germinability, such that the strength of the RT effect varied between taxa (Table 3b, Fig. 2).

Two best models were found for the effects of gut passage on germination time, and one for the effects of RT on germination time (Table S1). Germination time varied between taxa (Fig. 3) but was consistently reduced by gut passage for all taxa, with no significant Ttt*ST interaction (Table 3a). A significant ST*TR interaction showed that differences between the first and second trial varied between taxa, but all taxa germinated faster during the second trial (Fig. 3). RT had a curvilinear effect on germination time, although germination time decreased consistently with increasing RT over the range studied (Table 3b).

4. Discussion

At least some seeds of each studied taxa survived gut passage, suggesting that the great majority of plants in teal diet can be dispersed endozoochorously (see also Brochet et al., 2009, 2010). Small size was predicted to confer an advantage (more seeds excreted), since large seeds may become lodged within the gizzard until crushed, while smaller ones may pass through the gizzard more quickly (De Vlaming and Proctor, 1968; Figuerola et al., 2002). Soons et al. (2008) found in mallards that smaller seeds were retrieved in greater number, passed faster through the digestive tract and germinated better. In contrast, our results suggest that seed size *per se* has little influence on the probability of endozoochory by teal. We may have obtained different results if we had tested the same volume or biomass of seeds for each taxon instead of the same number, and fed them one taxon at a time as done by Soons et al. (2008).

In contrast to previous studies of *Chara* spp. (Proctor, 1962) and *S. maritimus* (Espinar et al., 2004), we found low germinability and

viability for both taxa, even for control seeds. Seeds may have been collected before they were fully mature, and many *S. maritimus* seeds were clearly empty. In the field, *Chara* spp. produce particularly large quantities of propagules (Jakobsson and Eriksson, 2000). As the proportion of seeds of a given species recovered from ducks increases with the number of seeds ingested (Figuerola et al., 2002), the proportion of *Chara* oospores surviving gut passage in the field was probably underestimated. Wild teal often have their oesophagus full of thousands of *Chara* oospores (Tamisier, 1971) and *Chara* was the most abundant taxon amongst viable diaspores in the lower gut of teal shot in the Camargue (Brochet et al., 2010).

Some of our results closely match findings under field conditions. For example, 78% of *P. pusillus* and 85% of *E. palustris* were viable after teal gut passage. Similarly, 73% *P. pusillus* and 94% of *E. palustris* found in the terminal part of wild teal guts were viable (Brochet et al., 2010). However, drying seeds for storage prior to the experiment may have enhanced dormancy and changed the hardness of seeds when compared to floating seeds or those from wet sediments. Our results may have changed if the experiment had been carried out immediately after seed collection, or if seeds had been stored wet. Although seeds are usually ingested in wet conditions in the field, Mediterranean wetlands and their seed banks are often subjected to drying in summer or in years of low rainfall (Espinar et al., 2005). Our experimental conditions may therefore best reflect those when reflooding occurs after a dry period.

The observed range of seed retrieval was of 2–63%, similar to the range of 2–48% observed by Holt Mueller and van der Valk (2002), 0–54% by Soons et al. (2008) and 2–51% by Wongsriphuek et al. (2008). Our captive teal had previously been fed on soft pellets rather than seeds, and this may have increased the proportion of retrieved seeds (Charalambidou et al., 2005). Birds in captivity have shorter intestines (Clench and Mathias, 1995), a change likely to accelerate the passage rate of seeds and reduce the number of seeds with long retention times (Traveset, 1998; Jordano, 2000). Survival of fennel pondweed (*Potamogeton pectinatus*) seeds after gut passage was roughly twice as high in mallards fed on soft pellets than in those fed on seeds, but germinability was not affected by diet (Charalambidou et al., 2005). Thus, unnaturally high values for the number of retrieved seeds may have been found, but the germinability and viability of evacuated seeds is not likely to have been strongly influenced by the captive diet.

Germinability and germination rate were generally increased after gut passage when compared to control seeds, although the strength of these effects varied between taxa. Acceleration of seed germination following gut passage has frequently been recorded in waterfowl, due to the scarification of seeds in gut passage (De Vlaming and Proctor, 1968; Figuerola et al., 2005), which uplifts the mechanical dormancy, making the seed coat permeable to water (Santamaría et al., 2002). Early germination following gut passage can have major consequences for fitness (Figuerola and Green, 2004). However, these effects are not consistent between studies. For instance, Soons et al. (2008) and Wongsriphuek et al. (2008) found germinability of some species to be reduced by gut passage. These authors stratified seeds at cold temperatures to break physical dormancy prior to the first germination runs. We chose to study the effect of gut passage both before and after stratification. For two species (*E. crus-galli* and *P. lapathifolium*), significant increases in germinability only became apparent after stratification, indicating that they have both mechanical and physical dormancy. Striking increases in germinability of *P. nodosus* and *P. pusillus* after stratification show that these taxa also have strong physical dormancy. The interaction between scarification by gut passage and winter stratification appears to be complex and species specific. Furthermore, the influence of gut passage on germination can vary greatly according to

salinity, which varies widely in Mediterranean wetlands (Espinari et al., 2004).

To our knowledge, this is the first experimental waterbird study to compare the effects of gut passage and retention time on both seed germinability and viability as indicated by the tetrazolium test. Gut passage increased viability for all taxa except *E. crus-galli*. Non-viable seeds may be preferentially digested, being more easily destroyed by physical and chemical action. Thus, observed increases in germinability after gut passage (Table 2) are not due exclusively to breakage of dormancy, but also to an increase in viability. Unlike previous studies in which germinability was tested but not viability (e.g. Soons et al., 2008; Wongsriphuek et al., 2008), we were able to separate these two effects and to analyze the germinability of viable seeds instead of visually-intact ones.

Similar to previous studies, most seeds were recovered within eight hours of ingestion (Holt Mueller and van der Valk, 2002; Soons et al., 2008; Wongsriphuek et al., 2008), and viability decreased with retention time (Pollux et al., 2005). This suggests that short distance dispersal is more frequent than long distance. Within their Camargue wintering quarter, teal usually fly a few kilometres (Tamisier and Dehorter, 1999; Guillemain et al., 2008). Thus, the tens of thousands of teal are effective dispersal agents for many wetland plant species at the local scale. Teal also show high regional mobility during winter, regularly moving into and out of the Camargue (Pradel et al., 1997; Lebarbenchon et al., 2009), and often switch wintering quarters in response to weather conditions (Ridgill and Fox, 1990). Furthermore, as some viable seeds were recovered at 48 h, long-distance dispersal can occur during teal migrations (Clausen et al., 2002; see also Brochet et al., 2009). Moreover small numbers of viable seeds were probably excreted after the experiment finished, since the maximum retention time in teal is known to be at least 60 h (Pollux et al., 2005). Overall, endozoochorous transport by teal appears to be a widespread and successful dispersal mechanism among wetland plants over a wide range of distances.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aquabot.2010.04.001.

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Supplementary data

Table S1. Null and best models ($\Delta AIC < 2$) of GLMMs for the proportion of germinated and viable seeds, and germination time for (a) treatment (Ttt, i.e. ingested versus control seeds) and (b) retention time (RT), ranked in a decreasing order of fit. The other variables are seed taxon (ST) and germination trial (TR). Null and best models are compared with analyses of variance.

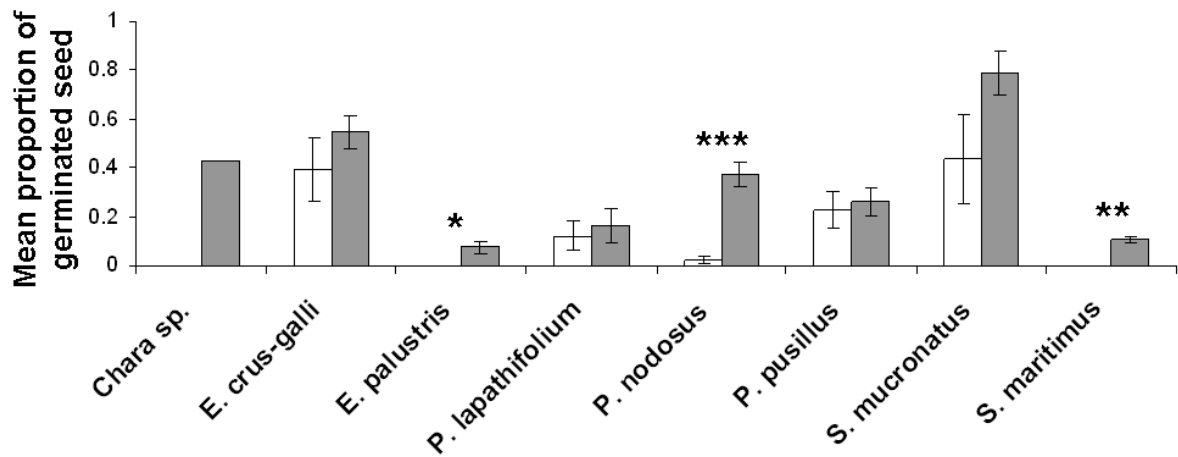
a.

Dependent variables	Models	AIC	ΔAIC	Model comparison
Germinated seeds	Ttt + ST + Ttt*ST	7626	0	$\chi^2 = 61$, df = 3,
	Null model	7681	55	p <0.001
Viable seeds	Ttt + ST + Ttt*ST	5207	0	$\chi^2 = 446$, df = 3,
	Null model	5648	441	p <0.001
Germination time	Ttt + ST+ TR + ST*TR	78114	0	$\chi^2 = 70630$, df = 5,
	Ttt + ST+ TR + Tttt*ST + ST*TR	78115	1	p <0.001
	Null model	148734	70620	

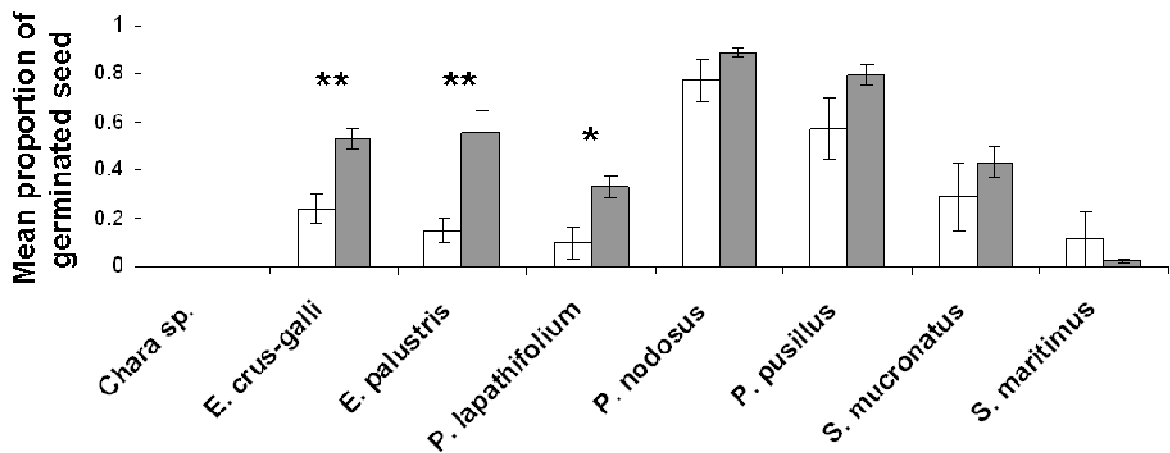
b.

Dependent variables	Models	AIC	ΔAIC	Model comparison
Germinated seeds	RT + RT ² + ST+ RT*ST	4350	0	$\chi^2 = 653$, df = 4,
	Null model	4996	646	p <0.001
Viable seeds	RT + RT ² + ST	4025	0	$\chi^2 = 981$, df = 4,
	RT + RT ² + ST + RT*ST	4025	0	p <0.001
	Null model	4999	974	
Germination time	RT + RT ² + ST + TR + ST*TR	88636	0	$\chi^2 = 6300$, df = 5,
	Null model	94899	6263	p <0.001

a.



b.



SUPPLEMENTARY FIGURE deleted from the final version on the Editor's

request. Mean proportion of germinated seeds after (a) the first and (b) the second germination trial for control (white) and ingested (grey) seeds, for each taxon (paired t-tests, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The proportion of germinated seeds was calculated in relation to the total number of viable seeds. Error bars indicate \pm standard-error for six replicates.