

Comparative dispersal effectiveness of wigeongrass seeds by waterfowl wintering in south-west Spain: quantitative and qualitative aspects

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Summary

1 The effectiveness of a seed disperser depends on the quantity of seed dispersed and the quality of dispersal provided to each seed. Relationships between the quantity and quality components and their dependence on characteristics of the dispersers remain largely uninvestigated.

2 The effectiveness of different waterfowl species at dispersing seeds of *Ruppia maritima* was evaluated in a wetland in south-west Spain. Droppings were collected during autumn and spring waterfowl migrations and the number of seeds ingested (estimated from seed fragments), undigested and viable in germination trials were determined.

3 Ingestion by waterfowl enhanced the rate of germination and, for several duck species, it also had a positive effect on germinability. Both the presence of seeds in the diet and the effects of gut passage showed high interspecific and temporal variance. Some of the interspecific variation in dispersal quality was related to gut structure: species with heavier gizzards destroyed a higher proportion of seeds and undigested seeds ingested by species with more grit in the gizzard germinated better.

4 In the waterfowl community studied, the quantity and quality components of seed dispersal effectiveness were positively correlated across species.

Key-words: dispersal quality, dispersal quantity, effects of seed digestion by vertebrates, germination rate, germination speed, gut structure, seed dispersal, seed germination

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Introduction

Many freshwater wetlands are isolated habitats equivalent to ecological islands, although there tend to be similarities in composition of aquatic plant (Good 1953; Raven 1963) and invertebrate communities (Brown & Gibson 1983) between different catchment areas. Aquatic habitats present relatively low rates of endemism compared with other ecosystems, suggesting that plants and invertebrates have significant rates of dispersal between wetlands (Santamaría 2002). The possibility of such dispersal by waterbirds has long been recognized (Darwin 1859; Ridley 1930), but little is known about its frequency in the field (see reviews by Figuerola & Green 2002; Green *et al.* 2002), especially in comparison with the relatively well-documented dis-

persal patterns of many terrestrial plants by frugivorous birds (see review in Janzen 1983; Jordano 2000). Most aquatic plants do produce non-fleshy fruits (e.g. *Zannichellia*, *Ruppia*), or produce fruits with a small proportion of edible material (e.g. *Potamogeton*). Hence, dispersal may depend on the non-intentional ingestion of seeds by herbivores eating vegetative parts or by filter feeding species, or on a fraction of the seeds consumed by granivorous species surviving ingestion.

Successful internal dispersal of aquatic plants results from interactions between seed processing, disperser movements, biotic and abiotic influences on the survivorship of seeds and seedlings and the growth and reproduction of the resulting individuals. The complete sequence of steps has however, seldom been considered for any plant species (but see Jordano & Schupp 2000, references therein). Many of these variables are likely to differ between species of disperser, whose individual effectiveness can be defined as the contribution it makes to the future reproduction of a plant. The ideal

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measure of dispersal effectiveness is the number of new plants produced as a result of the actions of a particular disperser relative to the numbers produced by other dispersers or by seeds that are not dispersed (Schupp 1993). However, due to the difficulties inherent in estimating adult fitness, such a definition has never been applied in the field, and less complete approaches based on seed viability or seedling survival have been used (Reid 1989). Identification of the attributes of effective dispersers is crucial for progress in the understanding of plant–animal interactions (Izhaki *et al.* 1991; Jordano & Schupp 2000). Many questions remain to be investigated, notably how the different components of seed dispersal effectiveness covary between disperser species and how quantity- and quality-related components interact. Seed processing by birds affects the germinability and/or the rate of germination of seeds. A recent review concluded that enhancement of germination following seed passage through the digestive tract of vertebrates occurred twice as often as inhibition (Traveset 1998; see also Traveset & Verdú 2002). Differences in seed retention time in the digestive tract, or in the type of food ingested with the seeds, could partly explain the variation in seed responses among and within plant species, and also among disperser species (Traveset 1998). These effects on germination are thus likely to play a role in determining disperser effectiveness, particularly for waterfowl species that ingest seeds mixed with other food (e.g. vegetative plant parts or zooplankton).

Wigeongrass, *Ruppia maritima* (L.), is a submerged angiosperm that inhabits brackish coastal and inland saline waters and presents a world-wide distribution, including all the continents and many islands between 69° N and 55° S (Verhoeven 1979). Mechanisms of dispersal of *Ruppia* seeds are poorly understood. Agami & Waisel (1988) demonstrated that seeds can resist passage through the gut of fish. *Ruppia* seeds are often consumed by waterbirds (Gaevskaya 1966; Cramp & Simmons 1977; Green *et al.* 2002), but no previous

information is available for any species of the genus about dispersal by waterfowl.

Here, we evaluate different components of dispersal effectiveness by migratory waterfowl species (10 duck species plus the Eurasian Coot, *Fulica atra*) in south-west Spain, examining separately the importance of seed ingestion, seed digestion and germinability. Thus, we focus both on the quantity (estimated as the number of seeds per dropping) and two aspects of the quality (the proportion of seeds that pass the gut intact, and the proportion of intact seeds that actually germinate) component of dispersal (Schupp 1993) provided by each species. We expect a decline in seed availability for waterfowl consumption during the winter, due to reduced accessibility after seeds are shed from the plants in autumn and a decrease in density in the seed bank caused by germination, as well as ongoing consumption during winter and early spring. We thus hypothesized that the number of seeds dispersed during the autumn will be greater than during the spring.

This study represents a first step towards establishing the ecological role of waterfowl in aquatic plant dispersal dynamics, and assesses the components contributing to the effectiveness of the major dispersers in this system.

Materials and methods

STUDY AREA

The study was carried out in ‘Veta la Palma’, a modified marsh within the Doñana wetland complex (south-west Spain; 6°14′ W, 36°57′ N). Veta la Palma is largely managed for fish farming with *c.* 40 roughly brackish rectangular ponds of rectangular shape, each *c.* 100 ha in area (total surface 3125 ha), and also includes 4442 ha of untransformed, temporary marshes. The area is used by many aquatic birds during the winter (20 000 to 74 000 ducks and coots observed in censuses between November 1998 and February

Table 1 Numbers of ducks and coot counted in the study area in aerial surveys done on 6 November 1998 and 23 February 1999 and mean numbers (and range) counted during four monthly surveys over the period November 1998 to February 1999. Source: Equipo de Seguimiento de Procesos Naturales, Estación Biológica de Doñana, CSIC, unpublished data

Species	6/11/98	23/02/99	November 1998–February 1999
<i>Anas acuta</i>	2000	1900	4153 (1900–9280)
<i>Anas clypeata</i>	19270	6480	15495 (6480–23700)
<i>Anas crecca</i>	2800	650	6030 (650–15870)
<i>Anas penelope</i>	14380	0	5148 (0–14380)
<i>Anas platyrhynchos</i>	2900	2000	3815 (2000–5340)
<i>Anas strepera</i>	700	30	1018 (0–3340)
<i>Aythya ferina</i>	900	6270	3466 (900–6270)
<i>Fulica atra</i>	13360	2260	5565 (2260–13360)
<i>Marmaronetta angustirostris</i>	1	0	51 (0–165)
<i>Netta rufina</i>	400	0	308 (0–800)
<i>Tadorna tadorna</i>	0	292	226 (0–310)
Unidentified anatidae	16000	400	8350 (400–16000)
Total	72711	20282	53625 (20282–74345)

Table 2 Number of faecal samples examined for each waterfowl species during autumn and spring 1998–99. The total number of droppings examined, the number of droppings with *Ruppia* remains (1), and with undigested *Ruppia* (2), as well as the total number of undigested seeds counted (3) are given for each species and season

Species	Autumn				Spring			
	Total	(1)	(2)	(3)	Total	(1)	(2)	(3)
<i>Anas acuta</i>	17	13	5	79	34	31	26	558
<i>Anas clypeata</i>	40	35	22	382	31	28	12	108
<i>Anas crecca</i>	8	4	2	4	22	15	0	0
<i>Anas penelope</i>	23	5	1	4	5	3	0	0
<i>Anas platyrhynchos</i>	62	32	7	25	63	58	39	957
<i>Anas strepera</i>	21	14	6	24	–	–	–	–
<i>Aythya ferina</i>	17	13	2	2	1	1	1	12
<i>Fulica atra</i>	92	54	20	87	70	13	4	6
<i>Marmaronetta angustirostris</i>	10	5	1	3	–	–	–	–
<i>Netta rufina</i>	3	1	1	1	3	2	1	36
<i>Tadorna tadorna</i>	–	–	–	–	4	4	2	13
Total	293	176	67	611	233	155	85	1690

1999, see Table 1). Islands that are present in most of the ponds, are used by roosting waterfowl during the day. During the study period, most of the fowl wintering in Doñana (total area: 166.000 ha) were concentrated in the study area due to the scarcity of water elsewhere. *Ruppia maritima* is the most abundant macrophyte in the area. In the sediments, the mean density of seeds in 5-cm deep, 4.4-cm diameter cores was 0.10 seeds cm⁻³ (SE = 0.02, $n = 96$) in September 1999, and 0.05 (SE = 0.01; $n = 96$) in May 2000, suggesting a reduction in the availability of seeds in the area during the course of the winter (Wilcoxon signed rank tests = 509.5, $P = 0.007$).

DATA COLLECTION

Fresh droppings were collected from islands with roosting waterfowl (Table 2). Individuals were located by an observer with the aid of a telescope, making use of the elevated position offered by pond dykes, and kept in view while the collector reached the island to collect the droppings. We selected long but narrow (less than 1 m wide) roost sites to facilitate the location of individual ducks using natural vegetation or small wooden stakes placed on each island as reference points. The differences in specific size of the droppings between some species (see Bruinzeel *et al.* 1997 for an overview of waterfowl dropping sizes) allowed us to confirm the taxonomic identity of the individual that produced the dropping. Droppings were first collected between 3 November and 3 December 1998 (autumn samples), a period overlapping with the end of the autumn southward migration from Central and North Europe (García *et al.* 1989; Scott & Rose 1996). A second set of droppings (spring samples) was collected in the period 22–25 February 1999, when many birds were leaving the study area towards their northern breeding grounds.

Faecal samples (one dropping per sample) were stored in individual tubes in the refrigerator until

analysed, during the following few days. Given the large number of birds at the study site, all (or almost all) samples were likely to be from different individuals. We collected more than one sample at the same location but, to minimize the chances of collecting droppings from the same individual and thus introducing some dependence in the data set these samples come from different waterfowl species. Faeces were washed with tap water using a 0.5-mm sieve. The items remaining in the sieve were examined under a dissecting microscope, and plant seeds or invertebrate eggs were identified (see Figuerola *et al.* 2001a for a summary of the propagules of other species found in the droppings). In the case of *R. maritima*, we counted separately the number of intact seeds, the number of seeds with a fractured seed coat (hard endocarp) but retaining an intact seed content (embryo plus endosperm), and the number of seed-coat fragments. The first two category ('undigested' seeds) groups were stored wet (in Eppendorf tubes filled with a few drops of tap water) in the refrigerator and used to test the viability after ingestion by waterfowl.

Control seeds (i.e. not retrieved from droppings) were collected in November 1998 from the surface of two ponds used to collect the droppings; collecting the seeds directly from the plants would have required a much earlier sampling because seeds are shed before the arrival of migratory waterfowl. However, given the moderate seed production per plant and the size of the populations (thousands of individuals), they must have originated from a large number of individuals. The low number of ponds sampled for controls could represent a problem if differences in germination do occur among populations inhabiting different ponds. For example, local adaptation in germination characteristics has been described between populations of *R. maritima* differing in salinity, inundation regime and/or sediment type (Van Vierssen *et al.* 1984). However, given the homogeneity of the ponds (that have been constructed and are managed for the same purpose, fish farming, and thus share the same sediment type,

Table 3 Gut structure in waterfowl collected from Doñana (south-west Spain). Mean \pm SE and sample size (in parentheses) are given

	Gizzard mass (g)	Grit mass (g)	Intestine length (cm)
<i>Anas acuta</i>	18.1 \pm 3.7 (2)	0.47 \pm 0.24 (2)	–
<i>Anas clypeata</i>	8.8 \pm 0.4 (34)	1.05 \pm 0.10 (41)	213 \pm 5 (34)
<i>Anas platyrhynchos</i>	36.4 \pm 2.0 (11)	2.45 \pm 0.44 (11)	150 \pm 18 (3)
<i>Anas strepera</i>	25.8 \pm 3.3 (5)	2.84 \pm 0.56 (5)	102 (1)
<i>Aythya ferina</i>	41.9 \pm 6.6 (3)	2.17 \pm 0.56 (3)	–
<i>Fulica atra</i>	44.7 \pm 4.3 (27)	8.78 \pm 0.57 (28)	115 \pm 6 (12)

water source and hydrological regime), and the high likelihood of seed dispersal among ponds found here, which would constrain local adaptation, we do not believe this could have biased the results of the analyses. Seeds were stored in Eppendorf tubes half filled with tap water and kept in the refrigerator for later germination experiments. A second batch of control seeds was allowed to dry on a sheet of filter paper before storage in the refrigerator until tested for germination. Both dry and wet seeds were used because *R. maritima* is known to show low germination unless dormancy is broken by drought or, potentially, by gut passage (with limited effects of winter stratification; Van Vierssen *et al.* 1984; Koch & Seeliger 1988). Hence, germination of wet, dormant seeds would have provided a poor indicator of seed viability. Control and duck- or coot-ingested seeds were germinated in microtitre trays with each 3.5 mL cell filled with 2 mL of tap water, placed in germination rooms at 20 °C and in the dark, to maximize the germination of seeds (see Koch & Seeliger 1988, Koch & Dawes 1991 and Acosta *et al.* 1998 for the effect of salinity and temperature on germination of *R. maritima*). We used dark conditions because they had been previously found to enhance germination of the local populations of *R. maritima* and *R. drepanensis* (L. Santamaría, unpublished data). Seeds from larger samples were randomly distributed between adjacent cells, such that each received a maximum of 10 seeds. Germination was checked every 2–3 days, until no further germination was detected (42 days in autumn and 58 days in spring). At the same time, water was replenished and germinated seeds removed. The few seeds that became infected by bacteria and/or fungi (mostly those with a cracked seed coat) were immediately removed and the water of the cell was refreshed. We did not apply antibiotics because we considered that increased infection risk is one of the processes affecting seed viability following gut passage.

We were unable to collect fresh controls in spring, because seeds in the field could either be uningested or ingested and defecated during the winter by birds (or fish). We therefore used autumn-collected dry seeds as controls, because moderately long dry storage of *R. maritima* seeds resulted in very little variation in their initial germination characteristics, although they may not reliably represent the behaviour of non-ingested seeds in the field.

Seed-endocarp fragments recovered from the droppings were dried for 2 days at 70 °C and weighed to the nearest 0.1 mg. To estimate the minimum number of seeds represented by the fragments from each dropping, 26 seeds were deliberately broken under finger pressure, and the remnants sorted using a 0.5-mm sieve, and dried for 2 days at 70 °C, before weighing. The average dry mass of the fragments derived from one seed was estimated to be 4.1 mg (SE = 0.07, $n = 8$). The number of broken seeds in each dropping was then estimated from the total mass of the fragments extracted, rounding up to the next whole number (e.g. 0.2 rounded up to 1), because the presence of a unique fragment implies the ingestion of at least one seed.

To test the possible role of gut morphology in explaining interspecific differences in quality of dispersal, we studied gut morphology using dead birds (Table 3). Dead waterfowl were obtained from various sources: illegally shot birds confiscated by the police; birds arriving dead at recovery centres; and individuals that died following a toxic spill in the Doñana area (see Aparicio *et al.* 1998; Grimalt *et al.* 1999) or during an epidemic of avian bronchitis. The different origins of carcasses could introduce a source of heterogeneity in the data set, but as all species come from a diversity of sources, this would decrease the chances of detecting differences between species, making our analyses more conservative (rather than causing any observed variation). Although ill birds might have stopped feeding for days before dying, a process that could affect gut size, the rather drastic types of mortality (avian bronchitis, acute toxicity) make this unlikely; indeed, the guts of most of the birds examined contained food. Gizzards were opened to extract their contents. Grit was separated from food by decantation, dried at 40 °C to constant mass and weighed to the nearest 0.01 g. Empty gizzards were also weighed to the nearest 0.01 g. Intestine length was measured with a ruler to the nearest cm. Since the structure of digestive organs and quantity of grit ingested may change seasonally (Troost 1981; Whyte & Bolen 1985; Mateo 1998), we only considered data from birds collected between October and December (autumn sample) and February and April (spring sample). Unfortunately, the small sample collected in spring (10 individuals of four species) was not enough to allow separate analysis. The repeatability of species means was estimated as the intraclass correlation of the

measures obtained from different individuals in the autumn (see Lessells & Boag 1987; repeatability estimates are generally reduced both by measurement error and intraspecific variability). Estimates of repeatability of species means were 69% for gizzard mass ($F_{7,96} = 26.74$, $P < 0.0001$), 85% for intestine length ($F_{4,50} = 44.78$, $P < 0.0001$) and 84% for grit mass ($F_{7,107} = 67.06$, $P < 0.0001$). Body mass for each species was estimated as the mean for male and female values from Figuerola & Green (2000) for ducks, and Cramp (1980) for coot. We did not obtain morphological data for all the species, so the number of species included in this analysis was lower than the total number of species for which seed survival and germination were measured.

STATISTICAL ANALYSES

To analyse the seasonal or species-specific patterns of variation in number of ingested seeds per dropping, we used general linear modelling (GLM). GLM allows a more versatile analysis of correlation than standard regression methods, because the error distribution of the dependent variable and the function linking predictors to it can be adjusted to the characteristics of the data. For these analyses we used a negative binomial error model with a log link function and type III sum of squares, implemented using the GENMOD procedure of the SAS program (v. 8.2, SAS Institute 2000). Poisson errors are widely used for the analyses of count data, but in our analyses they resulted in highly overdispersed models (Crawley 1993), making the negative binomial a more adequate error structure. The differences among species and seasons in the number of seeds surviving gut passage or the number of seeds successfully germinating were analysed with a GLM model with binomial error and a logit link function. This procedure uses the number of seeds ingested or the number used in the germination experiments as the binomial denominator, thus controlling for the effects of sample size on the proportion surviving gut passage or germinating (Crawley 1993). Deviances from the model were scaled with the square root of the ratio deviance/degrees of freedom to correct for the effects of data over-dispersion in the statistical test. For germination data, separate models were used for autumn and spring samples because, among other factors, the potential time for germination differed between trials. Unbiased estimates correcting for the effects of number of seeds per sample on the proportion of seeds surviving digestion or germinating were obtained by back-transformation of the estimates obtained from binomial GLM, and the standard error of these estimates was calculated using the Delta method (SAS Institute 1996).

When a factor with more than two levels was significant, the statistical significance of comparisons between levels of the factor was estimated by the likelihood ratio statistic (SAS Institute 2000). If the

interaction between factors was significant, we used the Wald chi-square test for differences between least-squares means (SAS Institute 2000).

To investigate the relationship between gut morphology and interspecific variation in seed ingestion, seed digestion and seed germinability, the factor 'species' was replaced by mean values of the different morphological characters considered for each species. The proportion of the interspecific variation explained was estimated, in separate models for each variable, as the ratio of the deviance explained by the morphological variable to that explained by the factor 'species'. The variable explaining most variance was then added to the model, and the significance of the remaining variables was tested again using type I sum of squares. Grit mass values were \log_{10} transformed before analysis.

The effects of seed ingestion on seed germination time were tested in a failure-time analysis by fitting a Cox proportional hazards regression model (e.g. Allison 1995) to data consisting of the number of days between sowing and seedling emergence, for each individual seed. Only data for seeds that had germinated by the end of the germination trials were included, in order to separate the effects on germination time from those on total germination. To account for the effects of digestion by different individuals (the 'dropping' effect, i.e. differences between faecal samples) or germination in different random groups (in the case of controls), a replicate effect was added to the model as a random or 'frailty' effect (MathSoft 1999). Bird species and control treatments were analysed as fixed effects. Ties were estimated using the exact method, in the program S-Plus 2000 (Mathsoft 1999).

The number of seeds ingested per dropping was considered as an estimate of the quantity component of dispersal, whereas the quality component of dispersal was estimated as the proportion of successfully germinating seeds compared with the total ingested. This assesses both the ability of seeds to pass through the gut without damage, and then to germinate. The relationship between the quantity and quality components of dispersal was first tested interspecifically by analysing the relationship (using Spearman rank correlation) between the estimates obtained from models analysing variation in quantity of seeds ingested vs. the proportion of these seeds successfully germinating. A second intraspecific analysis constructed separate GLM models for each species and season, analysing the relationship between quantity of seeds ingested (as the independent variable) and the proportion of seeds germinating (germinated/ingested as the binomial dependent variable).

To avoid pseudo-replication, the faecal sample (rather than the seed) is our unit in all analyses, as seeds coming from the same dropping are not likely to constitute independent samples. The number of species differed between analyses because of insufficient faecal samples containing seeds for some species.

Table 4 Mean number \pm SE and range of ingested seeds per dropping ('Ingested'), undigested seeds per dropping (i.e. seeds that retained intact the embryo and endosperm; 'Undigested'), and estimated proportion of undigested seeds that they represent (Undigested (%) = undigested/ingested). 'Estimated (%)' refers to the percentage of undigested seeds as estimated from the GLM models, which accounts for the differences in the number of seeds contained in each dropping (see Crawley (1993)). n = number of droppings examined. The number of ingested seeds is based on the total number of droppings examined, whereas both the number and the percentage of undigested seeds are based on the number of droppings with any seed remaining (thus the different n -values). Only species in which at least five droppings contained *Ruppia maritima* seed remains were included in these analyses

Species	Ingested \pm SE	Range	n	Undigested \pm SE	Range	n	Undigested (%) \pm SE	Estimated (%) \pm SE
Autumn samples								
<i>Anas acuta</i>	5.7 \pm 3.2	0–54	17	6.1 \pm 4.8	0–53	13	36.2 \pm 12.8	81.4 \pm 16.4
<i>Anas clypeata</i>	12.1 \pm 3.3	0–119	40	10.9 \pm 3.4	0–107	35	48.9 \pm 7.0	79.1 \pm 7.4
<i>Anas penelope</i>	0.4 \pm 0.2	0–5	23	0.8 \pm 0.8	0–4	5	16.0 \pm 16.0	44.4 \pm 54.0
<i>Anas platyrhynchos</i>	1.3 \pm 0.4	0–19	62	0.8 \pm 0.5	0–15	32	12.8 \pm 4.7	31.7 \pm 18.2
<i>Anas strepera</i>	2.0 \pm 0.7	0–10	21	1.7 \pm 0.8	0–9	14	27.1 \pm 9.3	57.1 \pm 25.0
<i>Aythya ferina</i>	0.9 \pm 0.2	0–3	16	0.2 \pm 0.1	0–1	13	6.4 \pm 4.8	12.5 \pm 40.5
<i>Fulica atra</i>	1.6 \pm 0.3	0–16	92	1.6 \pm 0.5	0–14	54	24.7 \pm 4.8	59.2 \pm 13.3
<i>Marmaronetta angustirostris</i>	0.8 \pm 0.4	0–4	10	0.6 \pm 0.6	0–3	5	15.0 \pm 15.0	37.5 \pm 57.2
Spring samples								
<i>Anas acuta</i>	50.3 \pm 4.9	0–122	34	18.0 \pm 4.2	0–85	31	24.7 \pm 3.8	32.6 \pm 8.7
<i>Anas clypeata</i>	9.7 \pm 3.8	0–102	31	3.9 \pm 2.3	0–65	28	24.4 \pm 5.9	36.0 \pm 20.7
<i>Anas crecca</i>	0.8 \pm 0.1	0–2	22	0 \pm 0	0	15	0	0
<i>Anas platyrhynchos</i>	29.9 \pm 4.4	0–136	63	16.5 \pm 4.0	0–132	58	31.6 \pm 4.5	50.8 \pm 8.3
<i>Fulica atra</i>	0.2 \pm 0.1	0–4	70	0.5 \pm 0.2	0–3	13	25.0 \pm 11.3	35.3 \pm 8.7

Results

THE QUANTITY COMPONENT OF *RUPPIA* DISPERSAL: PRESENCE OF *R. MARITIMA* IN THE DIET OF WATERFOWL IN AUTUMN AND SPRING

The number of ingested seeds per dropping differed between species (GLM $\chi^2 = 232.59$, 8 d.f., $P < 0.0001$, Table 4), and between seasons ($\chi^2 = 20.87$, 1 d.f., $P < 0.0001$). A significant interaction between the two factors was found ($\chi^2 = 140.94$, 3 d.f., $P < 0.0001$). When the two seasons were combined, *Anas acuta* and *A. clypeata* had the highest number of ingested seeds per dropping, *A. platyrhynchos* had significantly less, and *Fulica atra* had the least ($P \leq 0.05$ for all these contrasts). Other species could not be included in these comparisons because sufficient droppings were collected only in one season.

The comparisons within each season indicate that in autumn, *A. clypeata* droppings had the highest number of seeds (Table 4), significantly more than *A. acuta* ($\chi^2 = 3.53$, 1 d.f., $P = 0.06$), which had more seeds than the rest of the species ($\chi^2 \geq 21.13$, 1 d.f., $P \leq 0.0001$ for all the contrasts with other species). *A. strepera*, *A. platyrhynchos*, *F. atra* and *Marmaronetta angustirostris* all contained a small number of seeds, but significantly higher than *A. penelope*. In spring, interspecific differences in seed ingestion were more marked. *A. acuta* was the species with the highest number of seeds per dropping, and tended to have more than *A. platyrhynchos* ($\chi^2 = 3.37$, 1 d.f., $P = 0.07$). The droppings of both species presented significantly more seeds than those of *A. clypeata* ($\chi^2 \geq 14.45$, 1 d.f., $P < 0.0001$, for the two independent contrasts). Less seeds were found in

droppings of *A. crecca* ($\chi^2 \geq 6.74$, 1 d.f., $P \leq 0.009$ for all contrasts), and *F. atra* had the least seeds ($\chi^2 \geq 6.74$, 1 d.f., $P \leq 0.009$, for all contrasts).

Within bird species, the presence of seeds of *R. maritima* changed seasonally. Droppings of some species had more seeds in spring (*A. acuta*, $\chi^2 = 28.67$, 1 d.f., $P < 0.0001$; *A. platyrhynchos*, $\chi^2 = 143.93$, 1 d.f., $P < 0.0001$), one species had more in autumn (*F. atra*, $\chi^2 = 32.37$, 1 d.f., $P < 0.0001$), and *A. clypeata* showed no seasonal change ($\chi^2 = 0.46$, 1 d.f., $P = 0.50$).

THE QUALITY COMPONENT OF *RUPPIA* DISPERSAL: SEASONAL CHANGES IN THE BALANCE BETWEEN SEED PREDATION AND DISPERSAL

Overall, the proportion of undigested seeds in the droppings (Table 4) did not differ between species ($F_{6,292} = 1.57$, $P = 0.16$), but a significantly larger proportion of seeds survived digestion in autumn ($F_{1,292} = 5.49$, $P = 0.02$), and there was a significant species–season interaction ($F_{3,292} = 4.72$, $P = 0.003$). This interaction was related to the larger proportion of undigested seeds in autumn than in spring in the droppings of two species (*A. acuta*, $\chi^2 = 9.07$, 1 d.f., $P = 0.003$; *A. clypeata*, $\chi^2 = 17.57$, 1 d.f., $P < 0.0001$), and the lack of seasonal changes in other species (*A. platyrhynchos*, $\chi^2 = 1.40$, 1 d.f., $P = 0.24$; *F. atra*, $\chi^2 = 0.44$, 1 d.f., $P = 0.51$). When comparing estimates derived from ratios of undigested : ingested there seem to be seasonal differences also in *A. platyrhynchos*. However, these are due to seasonal changes in the abundance of seeds, not to seasonal changes in the proportion destroyed, as is made clear by examining the differences in the estimates obtained in the GLM

Table 5 Percentage and estimated percentage (\pm SE) of apparently viable seeds recovered from waterfowl faeces that germinated in autumn samples (after 42 days) and in spring samples (after 58 days). Percentage germinating was estimated from the ratio number of seeds germinating : number of undigested seeds in the dropping. Estimated percentage was estimated from the GLM models and accounts for the differences in the number of seeds contained in each dropping. Note that when the number of seeds is the same in all the samples (the case of controls) the germinability estimated directly from the sample and from the GLM model is the same. 'Samples (n)' refers to the number of droppings (undigested seeds) or the number of random germination batches (control seeds)

Autumn	Germinating (%)	Estimated percentage	Samples (n)	Seeds (n)
<i>Anas acuta</i>	17.6 \pm 9.1	8.9 \pm 16.3	5	77
<i>Anas clypeata</i>	50.8 \pm 8.1	19.9 \pm 7.4	22	217
<i>Anas platyrhynchos</i>	46.7 \pm 21.1	44.0 \pm 28.9	6	16
<i>Anas strepera</i>	25 \pm 17.1	12.5 \pm 29.5	6	25
<i>Fulica atra</i>	29.2 \pm 8.6	40.2 \pm 15.5	20	87
Dry control*	50.0 \pm 5.2	50.0 \pm 14.4	10	100
Wet control*	8 \pm 3.7	8.0 \pm 20.4	5	50
Spring				
<i>Anas acuta</i>	30.8 \pm 7.0	30.6 \pm 14.9	26	558
<i>Anas clypeata</i>	19.0 \pm 9.2	21.8 \pm 31.5	12	124
<i>Anas platyrhynchos</i>	25.5 \pm 5.9	32.1 \pm 11.4	38	955
<i>Fulica atra</i>	0	0	4	6
Dry control*	58.4 \pm 6.8	58.4 \pm 35.1	10	100

*Seeds from the plant, stored in dry or wet conditions (see Methods).

model that correct for the effect of differences in the denominator of the ratio (see Table 4). In the autumn, less seeds in droppings of *A. platyrhynchos* were undigested than in *A. clypeata* ($\chi^2 = 8.21$, 1 d.f., $P = 0.004$) and *A. acuta* droppings ($\chi^2 = 5.28$, 1 d.f., $P = 0.02$), whereas in spring the proportion was higher in *A. platyrhynchos* than in *A. acuta* ($\chi^2 = 15.87$, 1 d.f., $P < 0.0001$). None of the other comparisons within a given season was significant. In summary, therefore, this balance showed seasonal variation and better dispersal in autumn only for some waterfowl species.

THE QUALITY COMPONENT OF *RUPPIA* DISPERSAL: GERMINATION OF INGESTED SEEDS

In the autumn samples, total seed germination (after 42 days) differed significantly among species ($F_{6,68} = 5.04$, $P = 0.0003$, Table 5), with post-hoc tests identifying two groups. *A. acuta*, *A. strepera* and control (non-ingested) seeds, when stored wet, showed lower germination than the second group, which included *A. clypeata*, *A. platyrhynchos*, *F. atra* and the group of control seeds stored dry. However, in spring no significant differences were detected in the germination of undigested and non-ingested (control) seeds ($F_{4,85} = 0.77$, $P = 0.55$).

The comparison of germination patterns for undigested and control seeds indicated that undigested seeds germinated earlier than dry control seeds both in autumn ($\chi^2 = 24.37$, 1 d.f., $P < 0.0001$) and spring ($\chi^2 = 5.28$, 1 d.f., $P = 0.02$). In autumn, wet control seeds showed an intermediate germination time (which did not differ significantly from either dry control

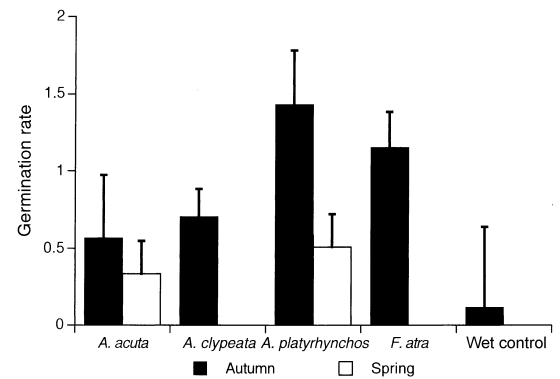


Fig. 1 Germination time, expressed as the parameter estimates obtained from separate Cox regressions for autumn and spring *Ruppia maritima* germination data. Bars correspond to model coefficients ($+1$ SE). A larger coefficient indicates faster seed germination. The coefficients for dry control seeds were set to zero. As data come from different germination trials and regression curves, coefficients for autumn and spring are not directly comparable. No spring data were available for *A. clypeata*, *F. atra* and wet controls.

($\chi^2 = 0.05$, 1 d.f., $P = 0.83$) or undigested ($\chi^2 = 1.86$, 1 d.f., $P = 0.17$) seeds).

Amongst autumn samples, seeds from droppings of *A. platyrhynchos* and *F. atra* germinated at a higher rate than seeds ingested by other species and controls (Fig. 1). Seeds from *A. clypeata* droppings germinated later but still at a higher rate than dry control seeds ($\chi^2 = 15.45$, 1 d.f., $P < 0.0001$). *A. acuta* seeds showed an intermediate germination time, not significantly different from any of the other species or the controls ($\chi^2 \leq 3.01$, 1 d.f., $P \geq 0.08$ for all the contrasts). Amongst spring samples, seeds from *A. platyrhynchos* droppings germinated at a higher rate than control

seeds ($\chi^2 = 5.85$, 1 d.f., $P = 0.02$), and the germination time of seeds from *A. acuta* droppings was intermediate and not statistically different from those of *A. platyrhynchos* ($\chi^2 = 0.79$, 1 d.f., $P = 0.37$) or control seeds ($\chi^2 = 2.43$, 1 d.f., $P = 0.12$).

INTRASPECIFIC AND INTERSPECIFIC VARIABILITY IN QUANTITY AND QUALITY OF DISPERSAL

When analysing the proportion of ingested seeds that germinate successfully (i.e. combining the effects due to mechanical seed destruction and the more subtle effects on germination ability), we again detected significant interspecific differences in autumn ($F_{3,153} = 3.58$, $P = 0.004$), and a less marked effect in spring ($F_{4,139} = 2.55$, $P = 0.04$). In autumn the best dispersal, in terms of the probability of an individual seed germinating was provided by *F. atra*, *A. clypeata* and *A. platyrhynchos*; the worst by *Ay. ferina*, *A. acuta* and *A. strepera*. In spring *A. platyrhynchos* was a better disperser than *A. clypeata* ($F_{1,139} = 5.94$, $P = 0.02$) and *A. acuta* ($F_{1,139} = 4.82$, $P = 0.03$), and no seed germinated successfully from *A. crecca* and *F. atra* droppings.

The relationship between the quantity and the quality components of dispersal was analysed by determining how the proportion of seeds that germinated is related to the number of seeds ingested by each waterfowl species. For these analyses we used the parameter estimates obtained from the models above. The parameter estimates for the proportion of seeds ingested that germinated presented a marginally significant positive correlation with the estimates obtained when analysing the number of seeds ingested by each species in each season (Spearman rho = 0.58, $P = 0.06$, $n = 11$ species \times season).

The results of analyses of the relationship between quantity and quality of dispersal at the intraspecific level varied among species and seasons. In two cases, the quantity of seeds ingested was not specifically related to the proportion of ingested seeds that germinated successfully (spring samples for *A. acuta* and *A. clypeata*, see Table 6). However, for three other cases, a significant positive relationship between seeds ingested and viability was found (autumn samples of *A. clypeata* and *F. atra*, and spring samples of *A. platyrhynchos*, Table 6).

Table 6 Relationship between the estimated quantity of *Ruppia maritima* seeds ingested and the proportion of the ingested seeds that germinated, given separately for each waterfowl species (i.e. comparing faecal samples within species). Estimates correspond to the slopes obtained from the GLM models, with binomial error and a logit link

Species	Season	Estimate \pm SE	<i>F</i>	d.f.	<i>P</i>
<i>Anas acuta</i>	Spring	0.015 \pm 0.009	2.36	1,29	0.14
<i>Anas clypeata</i>	Autumn	0.011 \pm 0.003	11.35	1,33	0.002
	Spring	-0.020 \pm 0.012	3.65	1,25	0.07
<i>Anas platyrhynchos</i>	Spring	0.012 \pm 0.005	5.26	1,56	0.03
<i>Fulica atra</i>	Autumn	0.157 \pm 0.045	14.06	1,52	0.0004

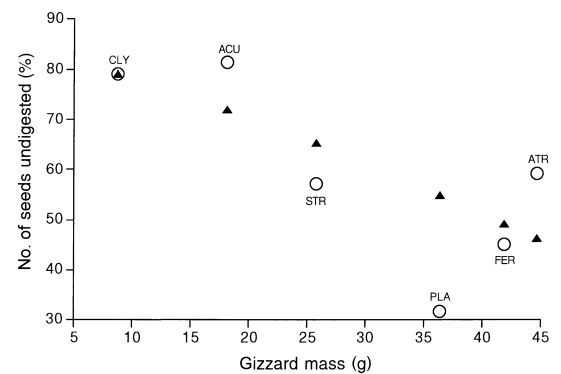


Fig. 2 Relationship between gizzard mass and estimated proportion of *Ruppia maritima* seeds surviving ingestion. Filled triangles show the values predicted for each species according to a GLM model and open circles the values observed for each species. Codes correspond to the first three letters of the specific Latin name.

SEED DISPERSAL EFFECTIVENESS AND ITS RELATION TO GUT MORPHOLOGY

Of the four anatomical traits considered (body mass, gizzard mass, grit mass and intestine length, Table 3), gizzard mass best explained the interspecific differences in the proportion of seeds undigested after gut passage (estimate \pm SE = -0.04 ± 0.01 , $F_{1,157} = 23.42$, $P < 0.0001$, Fig. 2). Although gizzard mass explained 61% of the interspecific variability, the categorical factor 'species' remained significant when added to the model ($F_{4,153} = 3.99$, $P = 0.004$). None of the other variables remained significant after controlling for gizzard mass ($P \geq 0.17$ for the other three variables).

Grit mass explained 73% of the interspecific differences in the proportion of undigested seeds that germinated successfully (1.22 ± 0.38 , $F_{1,58} = 10.26$, $P = 0.002$, Fig. 3). After controlling for grit mass, interspecific differences were no longer significant ($F_{3,55} = 1.25$, $P = 0.30$), nor were any of the other variables considered ($P \geq 0.50$). Grit mass also explained 36% of the interspecific differences in the proportion of ingested seeds that germinated (0.65 ± 0.26 , $F_{1,157} = 6.23$, $P = 0.01$). After controlling for grit mass, gizzard mass still explained a significant portion of interspecific variability (-0.03 ± 0.02 , $F_{1,156} = 4.50$, $P = 0.03$). In the model controlling for grit and gizzard mass, neither the factor 'species' ($F_{3,153} = 2.26$, $P = 0.08$) nor any of

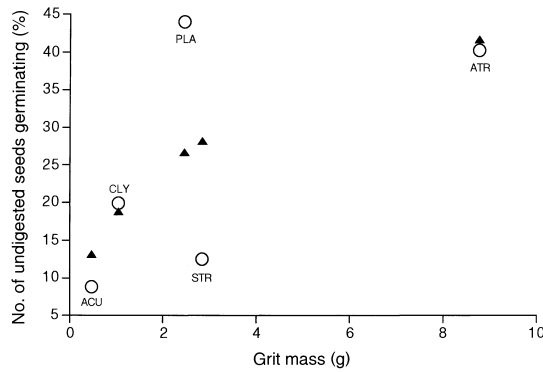


Fig. 3 Relationship between grit mass and the proportion of undigested seeds of *Ruppia maritima* germinating. Filled triangles show the values predicted for each species according to a GLM model and open circles the values observed for each species.

the other variables considered were significant ($P \geq 0.07$).

Discussion

Our results demonstrate the importance of waterfowl as dispersers of seeds of *R. maritima* at a local scale. There are high numbers of wintering waterfowl in the area and many seeds survive ingestion and can then germinate. The number of seeds transported within or through our study area each day by ducks and coots must be very high. This waterfowl mediated transport is likely to influence *R. maritima* population structure, given that one immigrant per generation seems enough to counteract population differentiation due to genetic drift (Wright 1951). However, the real impact of waterfowl on *R. maritima* population dynamics is difficult to evaluate without information on how successful dispersal translates into plant fitness. In any case, our results illustrate the potential importance of waterfowl-mediated transport for the colonization of new areas, or the re-colonization of temporary wetlands after periods of drought. Fish constitute another potential disperser of *Ruppia maritima*, given that a fraction of seeds can resist ingestion by some fish species and germinate in a higher proportion than control uningested seeds (Agami & Waisel 1988). However, unlike fish, waterfowl can potentially disperse the ingested seeds between hydrologically unconnected wetlands and even across isolated river basins. How fish and waterfowl differ in the quantity and quality components of *Ruppia maritima* dispersal is currently unknown.

Another important result of our study is the high potential for dispersal not only in early winter (when the seeds have recently been released from the plants), but also in early spring. This is a major difference with many previous studies on the dispersal of terrestrial fruiting plants, where dispersal is concentrated in the fruiting season (Izhaki & Safriel 1985). This difference is probably related to the filter feeding mechanism used by most waterfowl, that allows the ingestion of seeds

even when the birds are not feeding primarily on them (e.g. they may be extracted from sediments in which they have been deposited by ducks searching for benthic invertebrates). Orth *et al.* (1994) considered that waterfowl were not good candidates for the dispersal of *Zostera marina* because they arrived at the study area only after the period of seed release. However, our results indicate that this does not preclude dispersal by waterfowl, which can consume seeds several months after their production, thus making the examination of droppings necessary to evaluate the distribution of viable seeds.

We found marked interspecific and seasonal variation in the ingestion of seeds of *R. maritima* by waterfowl. The pattern of interspecific variation varied among seasons but, despite changes in their relative ranks, two species (*A. clypeata* and *A. acuta*) showed high seed consumption over both seasons. A high variability in the proportion of seeds destroyed during gut passage was also found. The proportion of seeds surviving gut passage was larger in autumn than in spring, but significantly so in the two species with high seed consumption. At least two non-exclusive factors may be involved. First, some species may have more developed gizzards in autumn than in spring (see below), thus destroying a larger fraction of the seeds ingested. Secondly, a larger fraction of seeds may have survived ingestion in autumn due to the large quantities ingested by some species in this season, and the positive relationship found by us between the quantity of seeds ingested and the proportion of viable seeds in the droppings. The effects of gut passage on seed viability also differed between species and seasons. As a consequence we found a high seasonal and interspecific variability in the potential for dispersal, a feature that characterizes bird-mediated seed dispersal dynamics in some terrestrial systems, and leads to non-equilibrium states of bird-plant interactions. Such conditions generally operate against mutual adaptations of interacting organisms (see Jordano 1994; Herrera 1998). Instability makes it difficult to infer selection pressures from field data, because the conditions of the system change both seasonally and among years. In our study system, only two species behaved consistently as a good (*A. clypeata*) or as a poor (*A. crecca*) disperser of *R. maritima*. The shoveler, *A. clypeata*, which preys mainly on invertebrates, is characterized by a highly specialized bill (Cramp & Simmons 1977). Seeds, which are accidentally ingested when filtering food, may pass undamaged through their gizzard, which, as characteristic of carnivorous species, is small and holds little grit (see Kehoe & Ankney 1985; Barnes & Thomas 1987). In addition to interspecific differences and seasonal changes in the rates of seed consumption, size of faecal droppings is an uncontrolled factor affecting our estimate of dispersal quantity. However, limited variation in dropping size and faecal output rate has been observed in four of the duck species used here (or can be expected for the others, given their similar size

and structure, e.g. dropping length ranged from 24.9 to 46.3 mm, see Appendix 1 in Bruinzeel *et al.* 1997) and there is much interspecific variation in the number of seeds dropping⁻¹ (mean seed abundance ranged from 0.4 to 12.1 in autumn samples and from 0.2 to 50.3 in spring). We therefore consider it very unlikely that this variability was explained only by differences in faecal output rate or dropping size.

In general, our study indicates a positive relationship between the quantity and quality components of dispersal in the waterbird guild studied. The species (and in many cases the individuals) consuming more seeds produced droppings with a higher proportion of viable seeds. Such a relationship has also been reported at the intraspecific level in *A. crecca* (Tamisier 1971). In terrestrial systems, an analysis with three species of birds also supported the existence of a positive relationship between quantity and quality components of dispersal (Larson 1991). Schupp (1993) also concluded that the quantity and quality components of dispersal were positively correlated, based on the reanalysis of data on dispersal of *Viola surinamensis* by six species of birds (see also Howe & van de Kerckhove 1981). However, this may not be the general case, as the two species of birds studied by Reid (1989) differed in the relative quality and quantity of dispersal provided to the plant. Clearly, more studies including a significant number of disperser species are necessary.

To our knowledge, this is the first study that documents differences in the viability of seeds after gut passage by most of the sympatric species of a given community of birds. In our case this consists mainly of species with close phylogenetic and ecological affinities (all Anatinae except for one rald). Previous studies have reported differences in viability after ingestion by disperser species belonging to different taxonomic classes (a deer and an iguana, Mandujano *et al.* 1994; a lizard and a bird, Nogales *et al.* 1998). Recently, Traveset *et al.* (2001) reported differences in the effect of ingestion of seeds by two species of passerine birds. Our study expands these results, showing that even closely related species (most were members of a single genus) can have very different effects on seed germination. These differences occurred both in autumn and spring, but as they varied over time, it is difficult to make generalizations.

What are the reasons for these interspecific differences? Differences in gut structure necessarily affect the treatment suffered by ingested food, and we found strong correlations between the gut structure of different species and digestive efficiency of ingested seeds. Species with heavier gizzards destroyed a higher fraction of the seeds (supporting the assumptions of previous work, e.g. Proctor *et al.* 1967), while the abundance of grit was positively associated with the proportion of seeds germinating after gut passage. The effects of gut ingestion on seed viability can be due to mechanical treatment in the gizzard and/or chemical treatment in the gut (Lohammar 1954; Teltscherova & Hejny 1973;

Sylber 1988). Our results indicate that the grinding effect is central to the effects of ingestion by birds on germination. A recent study in *Potamogeton pectinatus* concluded that the effects of ingestion by ducks upon germination are similar to those of scarification with sand, but completely different from those obtained after various periods of acid incubation (Santamaría *et al.* 2002; see also Spence *et al.* 1971). The apparent improvement in seed viability can also be explained by a selective destruction of seeds during gut passage, if for example larger seeds are more likely to resist ingestion (as larger seeds generally have higher germinability and shorter germination times; Greipsson & Davy 1995; Bond *et al.* 1999). However, smaller seeds seem more likely to survive ingestion by birds in both interspecific (De Vlaming & Proctor 1968) and intraspecific analyses (Figuerola *et al.* 2001b). Furthermore, in trials with captive ducks, Charalambidou *et al.* (2001) found no evidence for an effect of bird passage on *Ruppia maritima* seed viability due to size-related differences in destruction of seeds. In summary, our study shows that gizzard size has an important effect on the survival of seeds and that grit explains many of the effects of gut passage on viability.

Differences in the retention time of seeds in the gut are another possible reason for the observed differences in seed viability after ingestion by different waterfowl species. In captivity, we have found important differences between duck species in retention time and a significant effect of retention time on the viability of *Scirpus maritimus* seeds (Figuerola *et al.* 2001b). Together, these two effects may translate into interspecific differences between waterfowl in the viability of ingested seeds (Figuerola *et al.* 2001b; see also Traveset 1998). However, the effect of retention time upon viability cannot be generalized over species, as it was not significant in *Scirpus litoralis* (Figuerola *et al.* 2001b) or *Solanum americanum* (Wahaj *et al.* 1998). Although experiments in captivity showed that the viability of undigested *R. maritima* seeds varied with retention time, neither the retention time of seeds nor the patterns of variation in viability over retention time varied among duck species (Charalambidou *et al.* 2001). In our study, the relationship between intestine length and seed viability was weak and disappeared after controlling for the quantity of grit in the gizzard, suggesting that interspecific differences in retention time may be of minor importance. Body mass (an important correlate of intestine length, Herrera 1986; and of intensity of frugivory in terrestrial systems, Jordano 2000) was also a poor predictor of interspecific differences in seed viability.

Ingestion by waterfowl also accelerated germination, a frequent phenomenon in studies with birds (Traveset 1998). In autumn, the germination time of control seeds stored wet and dry did not differ, indicating that wet storage reduced germination time only when seeds undertook gut passage. The comparable germination time of dry and wet controls thus suggests

low seed coat permeability as one of the mechanisms responsible for seed dormancy in this species, and increased permeability following gut passage as one of the mechanisms that overcomes it. Although such an effect has often been considered beneficial for the plant, this remains unclear. Research on non-ingested seeds indicates that early germination can result in a competitive advantage over other seedlings (Zimmerman & Weis 1984; Waller 1985), but it can also increase seedling mortality due to exposure to harsh weather or pathogens (e.g. Traveset 1990). Clearly, the costs and benefits of early germination due to internal dispersal remain unevaluated, and experimental comparisons of the fate of ingested and control seeds would provide interesting results. In any case, ingestion by birds will diversify the germination response of seeds, a favourable process in environments with unpredictable climatic conditions (Harper 1977; Izhaki & Safriel 1990; Traveset *et al.* 2001).

The effects of seed passage through vertebrate guts on germination are not consistent among plant species (Traveset 1998). Given that temporal factors (changes in seed maturity or in disperser gut) can affect germination after passage, it is possible that this inconsistency is related to experimental conditions (see Figuerola & Green 2002, for a review of some problems associated with such experiments). As examples of seasonal changes in gut characteristics, Mateo (1998) reported an increase in quantity of grit ingested by *A. platyrhynchos* from October to March and no seasonal changes in *A. acuta*. However, gizzard mass decreased from autumn to early spring in *A. platyrhynchos* in Texas (Whyte & Bolen 1985), and increased in *A. strepera* in Louisiana (Paulus 1982). All this variability is likely to affect both the proportion of seeds surviving gut passage and their subsequent capacity to germinate.

In summary, waterfowl constitute an important dispersal agent for *Ruppia maritima*. Our results show that dispersal is not only concentrated during the seed production phase, but is also prolonged at least up to the end of the winter and early spring. Interestingly, potential for dispersal was found to show much seasonal and interspecific variation. Some of the interspecific variation was related to gut structure. The proportion of seeds destroyed during gut passage was related to gizzard mass and the quantity of grit was related to the germinability of seeds. Some of the seasonal variation could also be related to seasonal changes in gut morphology and/or grit consumption. Ingestion by waterfowl enhanced the rate of germination and, for several duck species, it also had a positive effect on the germinability. The quantity and quality components of seed dispersal effectiveness were positively correlated across waterfowl species, and species consuming more seeds also provided better dispersal for the plant (at least as measured by seed survival to ingestion and seed germinability). Examining other phases of dispersal could modify our estimates of effectiveness in the field. The spatial redistribution of the seeds due to waterfowl

transport can be an important factor affecting the seed rain shadows of *Ruppia maritima*, and possible differences in the conditions for germination and seedling survival among the sites where seeds are defecated by waterfowl would be an interesting topic for further research.

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