Bird-mediated seed dispersal: reduced digestive efficiency in active birds modulates the dispersal capacity of plant seeds

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Plant populations in fragmented ecosystems rely largely on internal dispersal by animals. To unravel the mechanisms underlying this mode of dispersal, an increasing number of experimental feeding studies is carried out. However, while physical activity is known to affect vertebrate digestive processes, almost all current knowledge on mechanisms of internal seed dispersal has been obtained from experiments with resting animals. We investigated how physical activity of the mallard *Anas platyrhynchos*, probably the quantitatively most important biotic dispersal agent in aquatic habitats in the entire Northern Hemisphere, affects gut passage survival and retention time of ingested plant seeds. We fed seeds of nine common wetland plants to mallards trained to subsequently swim for six hours in a flume tank at different swimming speeds (activity levels). We compared gut passage survival and retention times of seeds against a control treatment with mallards resting in a conventional dry cage. Intact gut passage of seeds increased significantly with mallard activity (up to 80% in the fastest swimming treatment compared to the control), identifying reduced digestive efficiency due to increased metabolic rates as a mechanism enhancing the dispersal potential of ingested seeds. Gut passage speed was modestly accelerated (13% on average) by increased mallard activity, an effect partly obscured by the interaction between seed retention time and probability of digestion. Gut passage acceleration will be more pronounced in digestion-resilient seed species, thereby modulating their dispersal distances. Our findings imply that seed dispersal potential by mallards calculated from previous experiments with resting birds is highly underestimated, while dispersal distances may be overestimated for some plant species. Similar effects of physical activity on digestive efficiency of mammals suggests that endozoochorous dispersal of plant seeds by vertebrates is more effective and plays a quantitatively more important ecological role in both terrestrial and aquatic ecosystems than previously thought.

Animal-mediated dispersal of plants is a wide-ranging phenomenon with an increasingly recognized ecological importance (Santamaría et al. 2007, Spiegel and Nathan 2007, Brochet et al. 2009, Schupp et al. 2010, Bauer and Hoye 2014). Especially dispersal by vertebrates (mostly birds, mammals and fish) has the potential to disperse large numbers of propagules over a wide range of distances, including long-distance dispersal, and even to disperse them disproportionally to suitable sites for establishment (directed dispersal, sensu Howe and Smallwood 1982). Hence, mechanistic understanding of the process of dispersal by vertebrates is highly relevant for understanding the dynamics of plant populations and ecosystems, and currently receives much attention in ecological research, through field studies, lab experiments and modelling, across a broad variety of ecosystems (Soons et al. 2008, Rodriguez-Perez et al. 2012, Spiegel and Nathan 2012, Viana et al. 2013b).

Animal-mediated dispersal may be of particular importance in ecosystems with a patchy distribution, where distinct units of suitable habitat occur scattered throughout an inhospitable matrix, such as freshwater wetlands which have been coined ecological 'islands' (Darwin 1909, Pimm and Raven 2000). The plant populations inhabiting such isolated habitats are particularly vulnerable to local extinction and depend critically on (directed) dispersal between habitat patches for local and regional persistence (Hanski 1998), adaptive capacity (Cousens et al. 2008) and expansion into new areas, for example following restoration activities (Verhoeven et al. 2008, Brederveld et al. 2011).

Charles Darwin was the first to point to waterbirds as potentially responsible for the large-scale dispersal of freshwater organisms (Darwin 1859). This hypothesis has gained much empirical support in recent years (Figuerola and Green 2002, Charalambidou and Santamaría 2002, Van Leeuwen et al. 2012b). Many species of waterbirds, but most prominently dabbling ducks, have been shown to transport viable plant propagules on their feathers or skin (epizoochory) or inside their digestive tract (endozoochory), both in experimental lab settings and in the field (Charalambidou and

Santamaría 2002, Figuerola and Green 2002, Soons et al. 2008, Brochet et al. 2010, Van Leeuwen et al. 2012b). Quantitatively, endozoochorous dispersal of seeds plays a vastly more important ecological role than epizoochory (Brochet et al. 2010, Costa et al. 2014). A review of diet studies in the seven dabbling duck species in the Western Palearctic showed that seeds of > 400 plant species are being consumed (Soons et al. unpubl.), many of which are likely to (occasionally) survive gut passage (Van Leeuwen et al. 2012b, Soons et al. unpubl.). With large numbers of waterbirds worldwide making regular local and migratory movements (Del Hoyo et al. 1992), millions of seeds of native and invasive species are estimated to be transported by waterbirds every day (Brochet et al. 2009, 2010). Mallards play a key role in the dispersal of plant seeds across the globe, due to their wide geographic distribution, high numbers, and opportunistic feeding behaviour (Soons et al. unpubl.).

To unravel the mechanisms underlying internal transport of plant seeds by waterbirds and quantify the role of this process in ecology, experimental feeding trials have been used extensively. In such experiments, resting, captive birds are fed a known quantity of propagules, which are then retrieved from their faeces collected at fixed time intervals (reviewed by Charalambidou and Santamaría 2002 and Van Leeuwen et al. 2012b). This provides essential information on the capacity of different propagules to survive gut passage, and the time it takes them to pass the digestive tract (retention time), thereby allowing calculations on dispersal effectiveness and potential dispersal distances (Charalambidou and Santamaría 2002, Figuerola and Green 2002, Soons et al. 2008, Guttal et al. 2011). As dispersal effectiveness and distance distributions (kernels) are crucial determinants of (spatial) population dynamics (Husband and Barrett 1996), data from feeding trials are often used to estimate these. Notably, dispersal distances are estimated based on feeding trial results, either by multiplying patterns of retention over time with theoretical vector speed (Charalambidou et al. 2003, Soons et al. 2008, Wongsriphuek et al. 2008), or by combining retention times with empirically determined vector movement patterns (Viana et al. 2013b).

Such estimations are extremely valuable for our understanding of the ecology of the dispersed organisms, but highly dependent on the accuracy of the experimental data. For practical reasons, most feeding trials to date have used animals resting in cages, particularly in the case of waterbirds (Santamaría et al. 2002, Charalambidou et al. 2005, Soons et al. 2008). However, in order to disperse propagules, animals have to move and physical activity is well known to affect digestive processes, probably due to reallocation of blood from the digestive organs to the muscles required for motion (Oettlé 1991, Brouns and Beckers 1993, Mortensen et al. 1998). This may greatly affect the applicability of results from feeding trials in calculating dispersal effectiveness and distances. Recently, Van Leeuwen et al. (2012a) found that plastic markers pass through the digestive tract of swimming mallards *Anas platyrhynchos* more rapidly and in higher numbers than in resting mallards. Indigestible plastic markers, however, do not accurately represent the natural situation, as they bypass the complex digestive processes that natural propagules encounter in the digestive tract. Hence, the aim of this study was to quantify how waterbird activity affects the retrieval pattern and dispersal potential of real, (partly) digestible plant seeds. To this end we performed a series of feeding experiments with mallards resting in a cage and swimming in a flume tank at different speeds. We expected that the increased activity levels induced by swimming would reduce the digestive efficiency and gut passage time. The result from this study allow a critical evaluation of the applicability of previous feeding trial studies for calculations on realistic dispersal effectiveness and distances in natural ecosystems.

Material and methods

To compare gut passage of plant seeds between resting and active waterbirds, eight adult female captive-bred mallards were subjected to feeding trials while resting and while floating or swimming. Before, after, and in-between trials the mallards were housed together in a free-range aviary on a commercial waterfowl diet of grains and pellets that were available ad libitum. During the trials, birds were kept individually allowing collection of their individual faeces. The feeding trials were conducted under five different conditions (hereafter 'treatments'): 1) resting in a dry cage (control, C), 2) floating in still water (S0), and 3–5) swimming in a flume tank at 0.2 m s^{-1} (S1), 0.4 m s^{-1} (S2) and 0.6 m s^{-1} (S3). While resting, each bird was in a conventional wooden dry cage (LWH: $0.54 \times 0.46 \times 0.48$ m) with a 12 mm mesh wire floor and front. These cages were placed side by side so the mallards were unable to see each other. While floating or swimming, the birds were put individually in a fixed rectangular mesh wire cage (LWH: $0.72 \times 0.41 \times 0.45$ m) on either side of the flume tank, again unable to see each other. In the oval shaped flume tank (LWH: $4.80 \times 2.05 \times 0.37$ m) two electric outboard motors were used to create a near-laminar water current with adjustable velocity. A more detailed description of this flume tank can be found in Van Leeuwen et al. (2012a).

During the five weeks before the experiment, the mallards were trained in the tank four times per week until they were physically fit to maintain a swimming speed of 0.6 m s^{-1} for 6 h. This maximum sustainable swimming speed was determined by gradually increasing the current velocity until the point where the mallards became unable to keep up by paddling and were pushed against the back of the cage. A maximum sustainable swimming speed of 0.6 m s^{-1} agrees well with that found in Prange and Schmidt-Nielsen (1970). The water current velocity was measured with an electromagnetic flow meter at approximately 10 cm under the water surface.

Experimental set-up

In a random block design each mallard received a single treatment (C, S0, S1, S2 or S3) per week until it had undergone all five treatments. Only in the last week of the experiment all mallards received the resting treatment at the same time, but none of the four flume tank treatments were ever given to more than two individuals per week. Each treatment started with force-feeding a mallard with a mix of 800 seeds of nine different species in three pill-shaped pellets of $\pm 40 \times 15$ mm that were held together by a thin layer of

dough (< 0.5 mm). Directly after feeding, the mallards were put in cages for their assigned treatments. During the resting treatments, mallard faeces were collected from a tray under the cage every hour for 12 h and once more after 24 h. Faeces sticking to the mesh wire were also collected. In the flume tank all faeces were caught in a nylon filter with 0.68 mm mesh behind each mallard and collected every hour. After 6 h, the mallards that were initially floating or swimming were also placed in the wooden cages. The trays under the cages were subsequently emptied every hour for 6 h (7–12 h after start of feeding trial) and once more at the end of the treatment, 24 h after force-feeding. Water was available for drinking ad libitum at all times, whereas food was unavailable during the 24 h trials to simulate fasting during flight. The mean water and air temperature during the experiments was 18.8 ± 0.9 and 20.6 ± 1.0°C respectively ($\pm$ SD). Collected faeces were carefully searched for intact seeds of the nine experimental species under a binocular microscope with a 10–$40 \times$ magnification.

Seed selection

Seeds of the nine freshwater wetland plant species used in all treatments belonged to nine different genera (Table 1) and were selected to represent a wide variation in seed size, but similarity in shape (spherical). A spherical shape had the advantages that seed volume was easily quantified using seed length, that this shape was representative for many seed species and that the seeds were well comparable with the round plastic markers used by Van Leeuwen et al. (2012a). One hundred seeds per species were fed per trial, except for two large-seeded species of which 50 seeds were fed, leading to a total of 800 seeds fed per mallard per treatment. Although the combined volume of seeds is in the upper range of volumes suggested for feeding trials in Van Leeuwen et al. (2012b), a mixture of seeds was preferred to avoid an effect of loading volumes on the digestive system. As seeds were all more or less spherical, seed length was used as a measure of seed size. Of each species 20 seeds were measured to the nearest 0.01 mm under a binocular microscope.

Statistical analysis

The typical data output of our experiment was, for each mallard in each treatment, the number of intact seeds of each plant species collected every hour after force-feeding for the first 12 h, and once more after 24 h. For analysis of the total

retrieved number of intact seeds we used all data over 24 h (model A). For analysis of retention time we used only retrieval data from the first 12 h (model B), as we were interested in the detailed retrieval pattern over time without large gaps.

We analysed the effect of treatment and seed size on the total retrieval of seeds and on the retrieval pattern over time (retention time and squared retention time) using repeated-measures generalized linear mixed-effects models (GLMM) with Poisson error distribution and log-link function. In model A we used summed total retrieval of intact seeds over 24 h per seed species as dependent variable. Treatment (C, S0, S1, S2 and S3) was included as fixed factor with the S0 (floating) treatment as reference level and seed size was included as centred covariate. We also tested for the interactive effect of treatment and seed size on total retrieval. In model B we used the number of intact seeds per seed species retrieved per hour as dependent variable, rather than average retention time, in order to preserve information about the pattern of retrieval over time. Treatment was included as fixed factor with the S0 treatment as reference level and seed size and retention time were included as centred covariates. To assess potential changes in retrieval patterns, the interactions of treatment with retention time and seed size with retention time (both linear and squared) were included. In both models (A and B) individual mallard was included as random intercept to correct for variation between individuals, and seed species was included as random intercept to avoid pseudo-replication due to multiple observations for equal seed sizes. We also tested a model with retention time as random slope within individual, but as this gave no different outcome we here report the results of the model with fixed slopes only, for reasons of parsimony.

Significance of terms and interactions was determined stepwise by likelihood ratio tests between models with and without terms of interest. Tukey HSD post hoc tests were used to analyse differences between treatments. Differences in seed size between species were tested with an ANOVA and Tukey HSD post hoc test. For all calculations we used the lme4 package (Bates et al. 2013) in R (<www.R-project.org>).

Results

Seeds of all species except AGR and IMP were retrieved intact from the faeces of mallards (Table 1), with four species (CAR, LOT, LYC and THA) retrieved intact in all treatments.

Table 1. Studied wetland plant seeds with their mean length, number of seeds fed to each mallard per treatment and overall mean intact retrieval.

Species	Family	Seed length (mm ± SD)	No. fed per treatment	Mean retrieval (%)
Persicaria bistorta (PER)	Polygonaceae	4.65 ± 0.36	100	0.01
Impatiens glandulifera (IMP)	Balsaminaceae	4.54 ± 0.59	50	0
Agrostemma githago (AGR)	Caryophyllaceae	3.37 ± 0.26	50	0
Sanguisorba officinalis (SAN)	Rosaceae	2.94 ± 0.41	100	0.18
Althaea officinalis (ALT)	Malvaceae	2.22 ± 0.15	100	0.33
Carex riparia (CAR)	Cyperaceae	2.17 ± 0.15	100	9.15
Thalictrum flavum (THA)	Ranunculaceae	2.06 ± 0.31	100	5.24
Lycopus europaeus (LYC)	Lamiaceae	1.36 ± 0.11	100	10.03
Lotus pedunculatus (LOT)	Fabaceae	1.18 ± 0.08	100	0.52

During three floating (S0) trials, two S1 trials and two S2 trials, a mallard regurgitated seeds and parts of feeding pellets, mixing regurgitated with defecated seeds and making further calculations related to gut passage impossible. Therefore, the results on these seven trials were omitted from further analysis in this study. In the remaining 33 successful feeding trials, retrieval of intact seeds already peaked 2–3 h after feeding and was low overall: mean retrieval per seed species ranged between 0 and 10.3% (Table 1). In the first 6 h (active phase) of the trials already 92.3% of all seeds were retrieved (Fig. 1a). Between 7–12 h after feeding 2.2% were retrieved and the last 4.7% of seeds were retrieved 13–24 h after ingestion. Variation in digestive efficiency between individual mallards was considerable, but all birds did excrete intact seeds in all trials. On average, total intact retrieval of all seeds pooled per individual per trial was 3.0% ($\pm$ 2.6 SD), ranging between 0.5% (C treatment) and 13.8% (S2 treatment).

Effect of vector activity

Total seed retrieval over 24 h was significantly affected by treatment (model A: $\chi^2 = 56.5$, DF = 4, p < 0.001; Fig. 1a, Table 2). Total retrieval of intact seeds was lowest in the resting (C) treatment (17.5 ± 3.6 SE seeds retrieved per mallard, 2.2% of total fed), but did not differ significantly from the floating (S0; 25.0 ± 6.7, ANOVA: Z = 2.4, p = 0.11) or S1 (22.3 ± 4.9, Z = 1.9, p = 0.30) treatments. In the S2 treatment, more than twice as many seeds were retrieved intact on average per mallard than in the resting treatment (37.2 ± 17.4), a highly significant difference (Z = 7.0, p < 0.001). Retrieval in S2 was also significantly higher than in S0 and S1 (Z = 4.3 and 5.0 respectively, p < 0.001). The mean number of seeds retrieved in the S3 treatment (27.6 ± 4.8) was slightly lower than in S2 (Z = –2.9, p = 0.03), significantly higher than C (Z = 4.5, p < 0.001) but not different from S0 (Z = 1.7, p = 0.40) and S1 (Z = 2.4, p = 0.11). These results suggest that swimming speed has an effect on retrieval of seeds, with more seeds being retrieved intact at higher activity levels, although this increase may be non-linear (Fig. 1a).

The hourly pattern of retrieval of intact seeds was also significantly affected by the interaction between treatment and both linear (model B: $\chi^2 = 45.3$, DF = 4, p < 0.001) and squared retention time ($\chi^2 = 46.0$, DF = 4, p < 0.001). This indicates that treatment also affects the retrieval pattern over time, although from Fig. 1b–c it becomes clear that
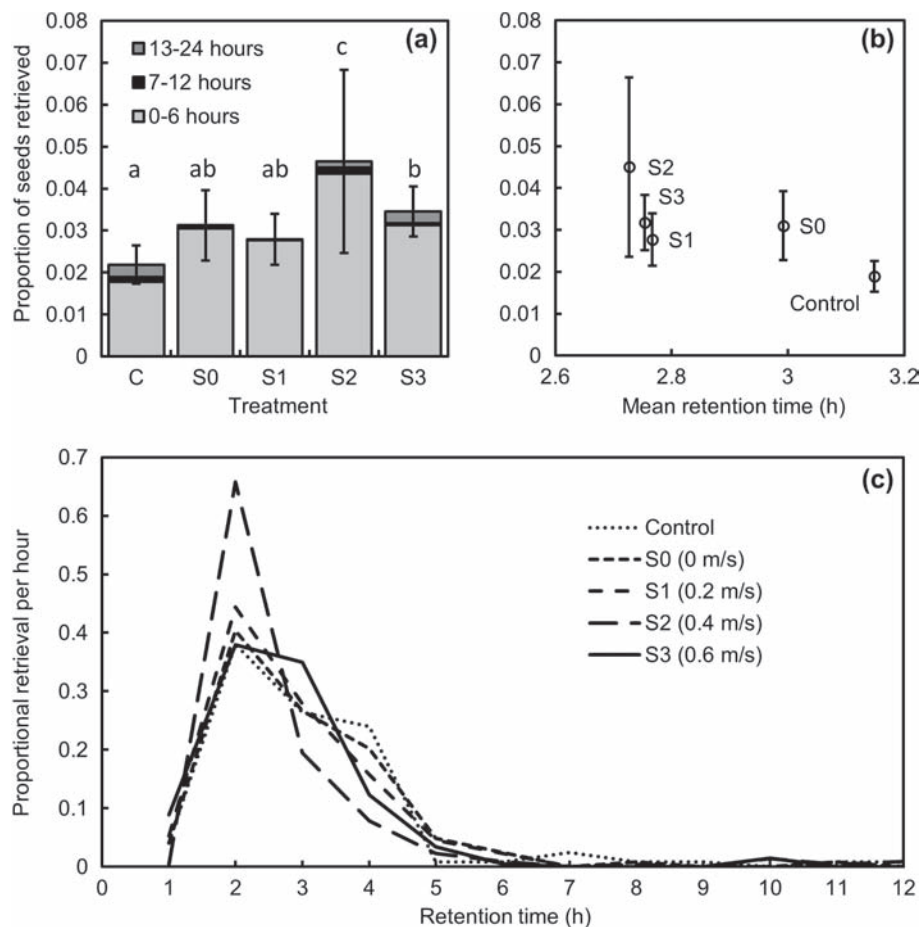

Figure 1. (a) Mean ($\pm$ SE) proportion of intact seeds (all species combined) retrieved per mallard per treatment in first 6 h (active phase), 7–12 h and 13–24 h. Letters indicate significant differences between treatments in retrieval over 24 h. (b) Relation between total proportion of seeds retrieved per treatment (mean per duck $\pm$ SE) and weighted mean retention time of retrieved seeds. (c) Retrieval pattern of all intact seeds combined over time for different treatments. Y-axis represents proportion of total number retrieved (not a proportion of fed seeds) within treatments.

Table 2. Contributions of terms and interactions to the tested models used to explain variation in retrieval of intact seeds. ΔAIC represents change in AIC upon removal of term/interaction from the model. χ^2-values and p-values of likelihood ratio tests are given. Dependent variables were total (summed) retrieval of ingested seeds over 24 h for model A and hourly retrieval of ingested seeds over 12 h for model B.

Model	Term/interaction	ΔAIC	χ^2	p-value
A	treatment	48.5	56.5	<0.001
A	seed size	5.4	7.4	<0.01
A	seed size : treatment	5.3	13.28	<0.01
B	treatment	29.5	37.6	<0.001
B	treatment : retention time	37.3	45.3	<0.001
B	treatment : retention time (squared)	37.9	46.0	<0.001
B	seed size	5.4	7.4	<0.01
B	seed size : treatment	−0.3	7.7	0.10
B	seed size : retention time	1.2	3.1	0.08
B	seed size : retention time (squared)	−2.0	0	0.99

this cannot simply be explained by a shift in the peak of retrieval. Indeed, mean retention time was not significantly different between treatments (ANOVA: F = 1.89, p = 0.15), although a clear trend is present with highest mean retention time in the control treatment (189 min) and the lowest mean retention time in the swimming treatments (163–166 min; Fig. 1b–c). Due to the skewed distribution of retention times, other parameters like median, 95% percentile and kurtosis are also informative, but none of these parameters differed significantly between treatments (median: F = 1.71, p = 0.19; 95% percentile: F = 1.41, p = 0.27; kurtosis: F = 0.664, p = 0.62).

Effect of seed size

Seed sizes of all studied plant species (Table 1) were significantly different from each other (p < 0.001) except for the combinations ALT-CAR-THA (p > 0.90), IMP-PER (p = 0.94) and LOT-LYC (p = 0.10). We found a significant negative effect of seed size on total retrieval (model A: $\chi^2 = 7.4$, DF = 1, p < 0.01, Fig. 2a), with seeds >3 mm rarely passing the digestive tract intact. The relatively low retrieval of the smallest species (LOT, Fig. 2a) deviates from this pattern and suggests that apart from seed size, other seed traits also play a role. We also found a significant effect of the interaction between treatment and seed size on total retrieval (model A, $\chi^2 = 13.28$, DF = 4, p < 0.01), suggesting that the effect of treatment on retrieval differs between seed sizes, but this difference does not appear to show any consistent pattern, and is small in comparison to the main effect of seed size (Fig. 2a). Seed size did not affect the retrieval pattern over time as indicated by the non-significant interactions with linear ($\chi^2 = 3.1$, DF = 1, p = 0.08) and square retention time ($\chi^2 = 0$, DF = 1, p = 0.99). The near-significant interaction between seed size and linear retention time supports a trend of lower retention times for larger seeds (Fig. 2b). Considering the low intact retrieval of large seeds, this probably indicates a rapidly increasing risk of digestion with longer retention time.

Discussion

Elevated activity levels in mallards caused an increased retrieval of intact seeds from the digestive tract, but did
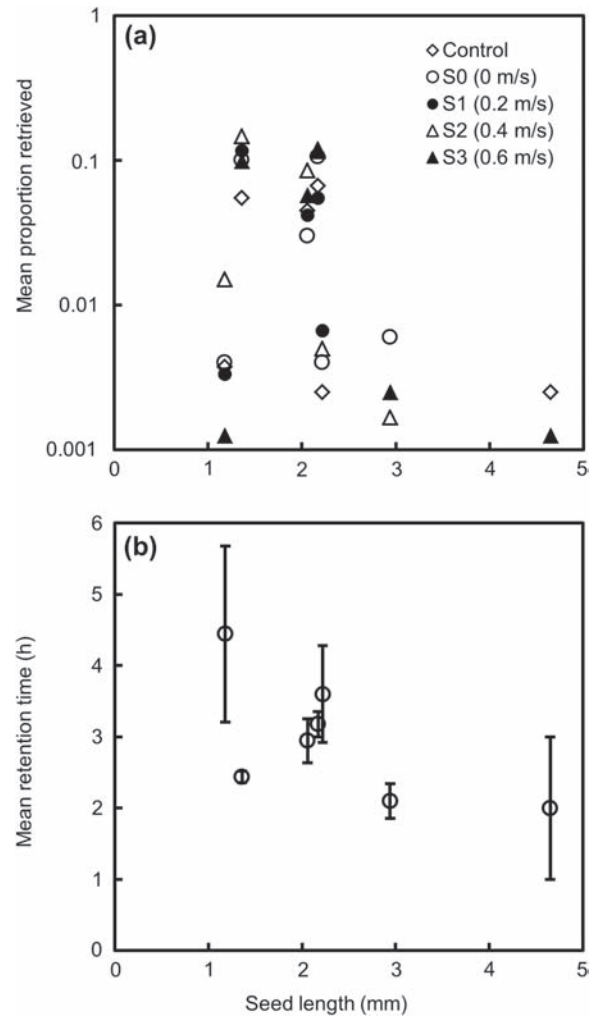

Figure 2. Effect of seed length on (a) mean proportional retrieval of intact seeds per mallard per treatment (note that Y-axis is on a log-scale) and (b) mean retention time. Two out of the nine seed species were never retrieved.

not cause a straightforward acceleration of gut passage of ingested propagules. This suggests that digestion efficiency itself is influenced by physical activity of mallards, an additional mechanism to earlier findings of accelerated gut passage of indigestible plastic markers due to increased physical activity (Van Leeuwen et al. 2012a). These findings imply that seed dispersal potential is enhanced in mallards that are actively moving across the landscape, in comparison to resting mallards.

Effects of physical activity and metabolic rate

Swimming, in general, clearly increased the total retrieval of intact seeds, compared to a resting state as used in all previous feeding experiments with mallards with real seeds. The level of mallard activity however did not show a clear linear relationship with seed retrieval, with the intermediate swimming speed increasing intact retrieval more than low and maximum swimming speeds. An elevated metabolic rate for thermoregulation to compensate for heat loss due to the high thermal conduction of water (when on the water in the flume tank versus sitting in a dry cage) may already contribute to the observed effect of swimming (Fig. 1a).

Prange and Schmidt-Nielsen (1970) found that thermal conductivity of water can already raise the metabolic rate of male mallards by 25–30%. The increased retrieval of seeds from floating mallards compared to mallards sitting in a dry cage, albeit not statistically significant, suggests that the metabolic rate of waterbirds, rather than their activity level per se, is the primary factor affecting propagule retrieval and retention times. This was also suggested by Van Leeuwen et al. (2012a), who found that indigestible plastic markers were retrieved earlier in floating than dry resting mallards. Their finding that gut passage was further accelerated in actively swimming mallards, indicated a combined effect of thermal conductivity and either an additional increase of metabolic rate or another physiological effect of physical activity (swimming). Moreover, Paladino and King (1984) found that heat increment of locomotion in birds might substitute for thermoregulation, resulting in a lower increase in metabolic rate in swimming birds compared to floating birds than expected from locomotion costs alone. This might partly explain the lack of differences in digestive efficiency between the wet treatments in this study. The effect of increased metabolic rate on the functioning of the digestive system might imply a prioritization of oxygen delivery to locomotion (or thermoregulation) on the expense of the digestive system (Hicks and Bennett 2004), indicating a 'locomotion-priority metabolic mode' which was also found in other taxa (McGaw 2007, Zhang et al. 2012). In literature on humans, the mechanism(s) that cause reduced transit times (Keeling and Martin 1987, Oettlé 1991) and increased stool weight (Coenen et al. 1992) under moderate exercise remain unclear, but gastrointestinal syndromes have been attributed to a redistribution of blood flow from the digestive system to the skeletal muscles (Ter Streege and Kolkman 2012).

By imposing different swimming speeds (and thereby inducing different metabolic rates, Prange and Schmidt-Nielsen 1970) we aimed at identifying a relationship between metabolic rate and digestive efficiency, which could be extrapolated to flight. Metabolic rates during flight are estimated to be almost three times higher than while swimming at maximum sustainable speed (Prange and Schmidt-Nielsen 1970, Nolet et al. 1992). However, as we found no conclusive support for a straightforward relationship between metabolic rate and digestive efficiency, our results do not allow direct extrapolation; we cannot exclude the possibility that this relation is non-linear, for example leveling off at higher metabolic rates. In the most extreme case, the prioritization of oxygen delivery to locomotion could, under heavy physical activity, lead to a temporal arrest of the digestive processes and even cause prolonged retention of food particles (McGaw 2007). It would be very relevant to clarify these mechanisms, because flying birds have a much higher long-distance dispersal capacity than swimming birds. Future studies with waterbirds flying in a wind tunnel may establish this.

We did not find a straightforward effect of mallard activity (or metabolic rate) on the speed of seed gut passage, although the highest mean retention times were in the control treatment and the lowest mean retention times in the swimming treatments. The overall effect of vector activity on the distribution of seed retention times appears to be relatively small, an effect likely to be caused by the inter-action between retention time and probability of digestion. We discuss this in the next section.

Digestible seeds versus indigestible plastic markers

The results of our study with real seeds demonstrate how vector activity modulates seed retrieval and hence dispersal potential. However, apart from the comparison between resting and active mallards, we can further improve our mechanistic understanding of the seed dispersal process under natural conditions by comparing our study to one in which indigestible plastic markers were fed to resting and active mallards (Van Leeuwen et al. 2012a). While we did not find a statistically significant shortening of the retention time of seeds with increasing mallard activity levels, this effect was very pronounced in a similar feeding experiment with indigestible round plastic markers of 2 mm diameter. In Van Leeuwen et al. (2012a), mean retention times of plastic markers during the first 12 h following ingestion were 6 h 30 min for resting, 5 h 55 min for floating and 5 h 20 min for swimming at maximum sustainable speed, compared to respectively 3 h 9 min, 3 h 0 min and 2 h 45 min in similarly sized seeds from our study (CAR, LYC and THA combined, Fig. 3). Hence, retention times of plastic markers were clearly longer, and appeared to differ more between treatments, than those of seeds. However, while the acceleration of gut passage from resting to fast swimming seems much larger for plastic markers (70 min) than for seeds (24 min), this corresponds to a relative acceleration of 18% and 13% respectively, which is remarkably similar. The acceleration of retrieval from floating to swimming birds was even more similar: 10% for plastic markers and 8% for seeds. The smaller relative acceleration for seeds is probably due to the positive relation between retention time and seed digestion. Digestible propagules retained longer in the digestive tract
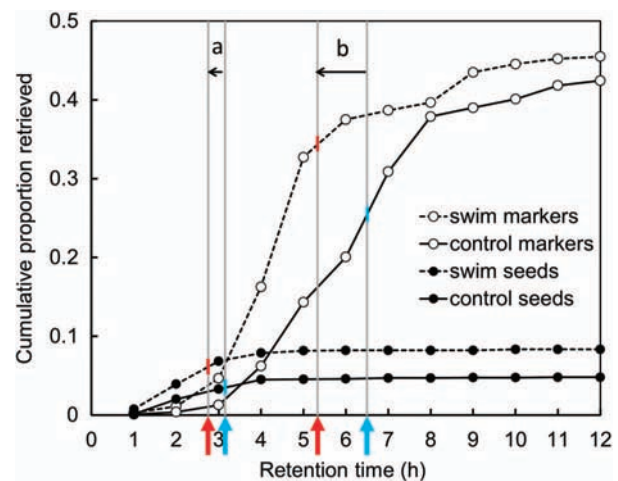

Figure 3. Cumulative retrieval patterns (as proportion of ingested) of digestible plant seeds (average of CAR, LYC and THA as used in this study; closed symbols) and indigestible plastic markers (open symbols) from faeces of mallards swimming at maximum sustainable speed (dotted line) and resting in a conventional dry cage (control; solid line). Arrows indicate the shift in mean retention time (the mean acceleration of retrieval) caused by the physical activity of the mallards, for seeds (a) and plastic markers (b). Data on plastic markers from Van Leeuwen et al. (2012a).

are more likely to be destroyed, ultimately resulting in low mean retention times. This mechanism partly obscures the effect of vector activity that is apparent for indigestible plastic markers. Hence, we conclude that increasing waterbird activity most likely speeds up seed gut passage, but that this effect is largely obscured in practice by the digestibility of seeds. The biological significance of this effect increases with more digestion-resilient propagules, which have longer mean retention times and will therefore experience more strongly accelerated gut passage times with increasing vector activity levels.

Effects of seed size

A negative relation between seed size and total intact retrieval was preserved in swimming mallards (Fig. 2a) and is in accordance with the mechanism proposed by Soons et al. (2008), that smaller seeds are more likely to escape mechanical digestive forces and hence have a higher survival of gut passage. However, the smallest species in our study (LOT) had a remarkably low retrieval, deviating from this relationship. We observed that seeds of LOT easily absorb water (a common trait in *Fabaceae* species), making them soft and vulnerable to mechanical stress. This effect has also been found in other *Fabaceae* species fed to cattle, where they survived very poorly (D'hondt and Hoffmann 2011). This emphasizes that seed size is not the only seed trait determining the potential survival of gut passage. While seed size may be the first determinant of gut passage survival across a wide range of species, crudely illustrating the mechanism of survival through escaping mechanical stress, seed coat characteristics have also been reported to affect gut passage (DeVlaming and Proctor 1968, Wongsriphuek et al. 2008) and differences between individual species may well be explained by additional seed traits such as water uptake and subsequent softening. The effects of seed size were consistent over treatments in our study, so we do not expect that conclusions from previous feeding experiments with resting waterbirds need re-evaluation in this regard.

Implications for dispersal

Results of this study imply that more seeds will survive gut passage in actively moving waterbirds than in resting ones, with a generally modest acceleration of gut passage (and perhaps consequent reduction of dispersal distance) depending on the digestion-resilience of the seed species. Smaller seeds survive gut passage in greater numbers than larger seeds, but this effect is not modulated by vector activity. These implications are based on the higher retrieval and slightly shorter mean retention times of intact seeds in actively swimming compared to resting mallards, as observed in our study. The total retrieval over 24 h for all seed species together was 58% higher at maximum sustainable swimming speed than in the resting treatment. For the three most retrieved (least digested) seed species this increase was even higher; intact retrieval of CAR, LYC and THA increased with 80%, 73% and 65% respectively. The modestly reduced retention times could further contribute to this increase, given the generally negative relationship between retention time and viability after gut passage (Charalambidou and Santamaría 2002).

Hence, vector activity appears to make effective dispersal more likely, but over somewhat shorter distances.

These effects of vector activity imply that estimates of dispersal probabilities and distances calculated from conventional feeding trials with resting mallards will underestimate dispersal probability for all seed species and may modestly overestimate dispersal distance for less digestible seed species. If we multiply seed retrieval over time, measured at the maximum activity level used in our study, with a flight speed of 75 km h^{-1} (Clausen et al. 2002), this results in maximum mean dispersal distances ranging between 174–211 km for seeds of $\pm$ 2 mm (CAR, LYC and THA). The dispersal distance distributions calculated using feeding trials with resting birds have overall lower dispersal frequencies but somewhat longer distances (Fig. 4). Although actual dispersal distances depend critically on the behaviour of the vectors (Kleyheeg et al. unpubl.), these estimated maximum distances are more than sufficient for seeds to reach isolated freshwater wetlands in a wide variety of landscapes. The fact that 92.3% of retrieved seeds in our study were retrieved in the first 6 h of the feeding trials, indicates that dispersal over more than 450 km is probably very rare, even though longer migratory flights of waterfowl are not uncommon (Viana et al. 2013a).

The fact that intact seeds of seven out of the nine plant species used in this study were retrieved, supports previous findings that there is a high potential among a wide range of different species of wetland plants for endozoochorous dispersal by mallards (Van Leeuwen et al. 2012b). Considering the large amount of seed species ingested by mallards, as demonstrated by gut-content-based diet studies (seeds of $>$ 400 plant species recorded in mallards in the Western Palearctic, Brochet et al. 2011, Soons et al. unpubl.), and given that mallards are the world's most abundant duck species with a very wide distribution (Del Hoyo et al. 1992), known to have frequent local (Legagneux et al. 2009, Sauter et al. 2012) and migratory flights (Viana et al. 2013a), we conclude that the results of our study further build support for the important ecological role of mallards. The effectiveness of mallards and other waterbirds in dispersing plants may have been underestimated based on earlier studies, and our increased mechanistic understanding of the dispersal process indicates that they may play an even more important role in plant population dynamics and structuring of (freshwater) ecosystems than previously considered (Green and Elmberg 2014).

Conclusions

Intact passage of plant seeds through the digestive tract increased with physical activity of mallards, by up to 80% in comparison to resting mallards. This is likely regulated through a reduced digestive efficiency with increased metabolic rate of the waterbirds, a mechanism enhancing the dispersal potential of ingested seeds. Calculations of dispersal efficiency and distance distributions (dispersal kernels) based on data from conventional feeding trials with resting captive waterbirds therefore likely produce underestimations of actual dispersal rates in the field, and should either be corrected for bird activity levels or discussed in relation to this. Physical activity did not simply reduce retention times of
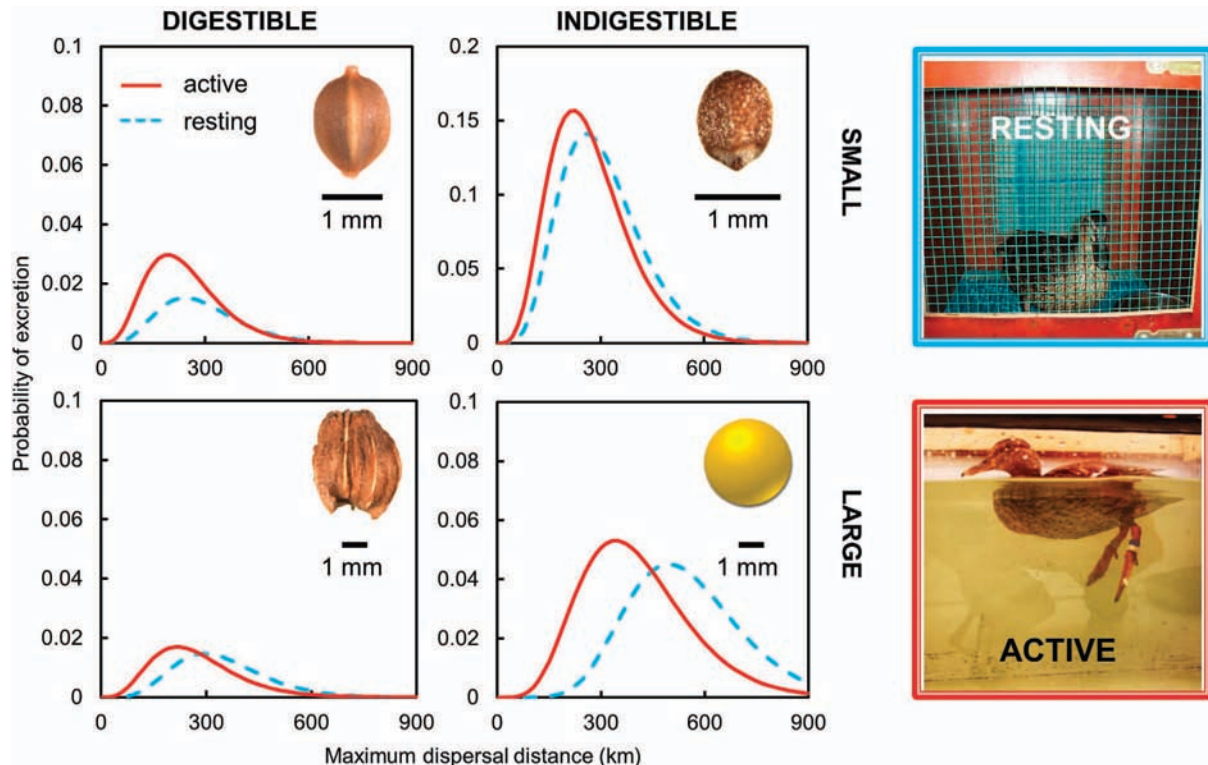
Figure 4. Conceptual diagram of dispersal kernels resulting from the effect of physical activity on digestive efficiency and retention time and the interaction with propagule size and digestibility. Graphs represent the estimated probability of excretion of ingested propagules over distance during a long unidirectional flight of a mallard at a flight speed of 75 km h^{-1}, deduced from retention curves from experiments with resting mallards (blue dotted lines) and mallards swimming at maximum sustainable speed (red solid lines). The reduction of retention time (and thus dispersal distance) with increased physical activity is most pronounced in large propagules, which already have a relatively long retention time, especially when they are resistant to digestion. A reduced digestive efficiency (1 – area under curve) is most pronounced in easily digestible propagules, although large, weak propagules will probably be digested either way. Photographs of seeds with permission from the Digital Seed Atlas (Cappers et al. 2006).

seeds in mallards, as the interaction between retention time and digestion partly obscures the effects of increased gut passage speed for digestible seeds (as opposed to indigestible plastic markers). An acceleration effect will be more pronounced in more digestion-resilient seed species, modulating their potential dispersal distances. The overall positive effects of metabolic rate on the dispersal potential of seeds found in this study imply an even more effective role of mallards and other waterbirds as seed dispersers, with a greater importance for spatial plant population dynamics and connectivity of (freshwater) ecosystems, than previous studies already suggested. The underlying mechanisms through which waterbird activity modulates dispersal effectiveness and distances may apply to other vertebrate disperser species as well, with implications for assessment of their role as seed dispersers.

Data accessibility

Data available from Dryad Digital Repository < http://dx.doi.org/10.5061/dryad.kt2g7 >.

References

Bates, D. et al. 2013. lme4: Linear mixed-effects models using Eigen and S4. R package ver. 1.0–5. – <http://CRAN.R-project.org/package=lme4>.

Bauer, S. and Hoye, B. J. 2014. Migratory animals couple biodiversity and ecosystem functioning worldwide. – Science 344: 54–63.

Brederveld, R. J. et al. 2011. Dispersal as a limiting factor in the colonization of restored mountain streams by plants and macroinvertebrates. – J. Appl. Ecol. 48: 1241–1250.

Brochet, A. L. et al. 2009. The role of migratory ducks in the long-distance dispersal of native plants and the spread of exotic plants in Europe. – Ecography 32: 919–928.

Brochet, A. L. et al. 2010. Plant dispersal by teal (*Anas crecca*) in the Camargue: duck guts are more important than their feet. – Freshwater Biol. 55: 1262–1273.

Brochet, A. L. et al. 2011. Is diet segregation between dabbling ducks due to food partitioning? A review of seasonal patterns in the Western Palearctic. – J. Zool. 286: 171–178.

Brouns, F. and Beckers, E. 1993. Is the gut an athletic organ? – Sports Med. 15: 242–257.

Cappers, R. T. J. et al. 2006. Digital aeed atlas of the Netherlands. Groningen Archaeological Studies, Vol 4. – Barkhuis Publishing and Groningen Univ. Library.

Charalambidou, I. and Santamaría, L. 2002. Waterbirds as endozoochorous dispersers of aquatic organisms: a review of experimental evidence. – Acta Oecol. 23: 165–176.

Charalambidou, I. et al. 2003. Effect of ingestion by five avian dispersers on the retention time, retrieval and germination of *Ruppia maritima* seeds. – Funct. Ecol. 17: 747–753.

Charalambidou, I. et al. 2005. Digestive plasticity in mallard ducks modulates dispersal probabilities of aquatic plants and crustaceans. – Funct. Ecol. 19: 513–519.

Clausen, P. et al. 2002. Long-distance endozoochorous dispersal of submerged macrophyte seeds by migratory waterbirds in northern Europe – a critical review of possibilities and limitations. – Acta Oecol. 23: 191–203.

Coenen, C. et al. 1992. Does physical exercise influence bowel transit-time in healthy young men. – Am. J. Gastroenterol. 87: 292–295.

Costa, J. M. et al. 2014. Endozoochory largely outweighs epizoochory in migrating passerines. – J. Avian Biol. 45: 59–64.

Cousens, R. et al. 2008. Dispersal in plants. – Oxford Univ. Press.

Darwin, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. – John Murray.

Darwin, F. 1909. The foundations of the origin of species. Two essays written in 1842 and 1844. – Cambridge Univ. Press.

Del Hoyo, J. et al. 1992. Handbook of the birds of the world, Vol 1: ostrich to ducks. – Lynx Edicions.

DeVlaming, V. and Proctor, V. W. 1968. Dispersal of aquatic organisms: viability of seeds recovered from the droppings of captive killdeer and mallard ducks. – Am. J. Bot. 55: 20–26.

D'hondt, B. and Hoffmann, M. 2011. A reassessment of the role of simple seed traits in mortality following herbivore ingestion. – Plant Biol. 13: 118–124.

Figuerola, J. and Green, A. J. 2002. Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. – Freshwater Biol. 47: 483–494.

Green, A. J. and Elmberg, J. 2014. Ecosystem services provided by waterbirds. – Biol. Rev. 89: 105–122.

Guttal, V. et al. 2011. Retention time variability as a mechanism for animal mediated long-distance dispersal. – Plos ONE 6: e28447.

Hanski, I. 1998. Metapopulation dynamics. – Nature 396: 41–49.

Hicks, J. W. and Bennett, A. F. 2004. Eat and run: prioritization of oxygen delivery during elevated metabolic states. – Resp. Physiol. Neuro. 144: 215–224.

Howe, H. F. and Smallwood, J. 1982. Ecology of seed dispersal. – Annu. Rev. Ecol. Syst. 13: 201–228.

Husband, B. C. and Barrett, S. C. H. 1996. A metapopulation perspective in plant population biology. – J. Ecol. 84: 461–469.

Keeling W. F. and Martin, B. J. 1987. Gastrointestinal transit during mild exercise. – J. Appl. Physiol. 63: 978–981.

Legagneux, P. et al. 2009. Variation in home-range size and movements of wintering dabbling ducks. – J. Ornithol. 150: 183–193.

McGaw, I. J. 2007. The interactive effects of exercise and feeding on oxygen uptake, activity levels, and gastric processing in the graceful crab *Cancer gracilis*. – Physiol. Biochem. Zool. 80: 335–343.

Mortensen, F. V. et al. 1998. Effects of moderate exercise on blood flow in the gastrointestinal tract in trained conscious miniature swine. – Digest. Surg. 15: 665–668.

Nolet, B. A. et al. 1992. Estimation of daily energy-expenditure from heart-rate and doubly labeled water in exercising geese. – Physiol. Zool. 65: 1188–1216.

Oettlé, G. J. 1991. Effect of moderate exercise on bowel habit. – Gut 32: 941–944.

Paladino, F. V. and King, J. R. 1984. Thermoregulation and oxygen consumption during terrestrial locomotion by white-crowned sparrows *Zonotrichia leucophrys gambelii*. – Physiol. Zool. 57: 226–236.

Pimm, S. L. and Raven, P. 2000. Biodiversity: extinction by numbers. – Nature 403: 843–845.

Prange, H. D. and Schmidt-Nielsen, K. 1970. The metabolic cost of swimming in ducks. – J. Exp. Biol. 53: 763–777.

Rodriguez-Perez, J. et al. 2012. Frugivore behaviour determines plant distribution: a spatially-explicit analysis of a plant–disperser interaction. – Ecography 35: 113–123.

Santamaría, L. et al. 2002. Effect of passage through duck guts on germination of fennel pondweed seeds. – Arch. Hydrobiol. 156: 11–22.

Santamaría, L. et al. 2007. Predicting spatial patterns of plant recruitment using animal-displacement kernels. – Plos One 2: e1008.

Sauter, A. et al. 2012. Individual behavioural variability of an ecological generalist: activity patterns and local movements of mallards (*Anas platyrhynchos*) in winter. – J. Ornithol. 153: 713–726.

Schupp, E. W. et al. 2010. Seed dispersal effectiveness revisited: a conceptual review. – New Phytol. 188: 333–353.

Soons, M. B. et al. 2008. Small seed size increases the potential for dispersal of wetland plants by ducks. – J. Ecol. 96: 619–627.

Spiegel, O. and Nathan, R. 2007. Incorporating dispersal distance into the disperser effectiveness framework: frugivorous birds provide complementary dispersal to plants in a patchy environment. – Ecol. Lett. 10: 718–728.

Spiegel, O. and Nathan, R. 2012. Empirical evaluation of directed dispersal and density-dependent effects across successive recruitment phases. – J. Ecol. 100: 392–404.

Ter Steege, R. W. F. and Kolkman, J. J. 2012. Review article: the pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchic blood flow. – Aliment. Pharm. Ther. 35: 516–528.

Van Leeuwen, C. H. A. et al. 2012a. Vector activity and propagule size affect dispersal potential by vertebrates. – Oecologia 170: 101–109.

Van Leeuwen, C. H. A. et al. 2012b. Gut travellers: internal dispersal of aquatic organisms by waterfowl. – J. Biogeogr. 39: 2031–2040.

Verhoeven, J. T. A. et al. 2008. An operational landscape unit approach for identifying key landscape connections in wetland restoration. – J. Appl. Ecol. 45: 1496–1503.

Viana, D. S. et al. 2013a. Migratory strategies of waterbirds shape the continental-scale dispersal of aquatic organisms. – Ecography 36: 430–438.

Viana, D. S. et al. 2013b. Allometric scaling of long-distance seed dispersal by migratory birds. – Am. Nat. 181: 649–662.

Wongsriphuek, C. et al. 2008. Dispersal of wetland plant seeds by mallards: influence of gut passage on recovery, retention, and germination. – Wetlands 28: 290–299.

Zhang, W. et al. 2012. The effect of dissolved oxygen levels on the metabolic interaction between digestion and locomotion in Cyprinid fishes with different locomotive and digestive performances. – J. Comp. Physiol. B 182: 641–650.