

# Consequences of intraspecific seed-size variation in *Sparganium emersum* for dispersal by fish

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## Summary

1. The potential for seed dispersal by fish (ichthyochory) is likely to vary within aquatic plant species, depending on intraspecific variation in phenotypic seed traits.

2. We studied the effect of seed size variation within the unbranched burreed (*Sparganium emersum*) on the potential for internal dispersal by the common carp (*Cyprinus carpio*), by feeding them light (< 10 mg), medium (10–20 mg) and heavy (> 20 mg) seeds, seed mass being positively related to seed size.

3. We hypothesized: (i) that ingestion, retention time, survival during gut passage and viability after gut passage of *S. emersum* seeds would be affected by seed size; and (ii) that this would translate into intraspecific variation in dispersal probability and dispersal distance among seed size categories.

4. Ingestion was negatively related to seed size, while survival during gut passage was positively related to seed size. Seed viability after gut passage was not affected by seed size. Since the negative effect of ingestion was counterbalanced by an equally strong but positive effect on seed survival, the probability of dispersal did not differ between the tested seed-size categories.

5. The time that seeds remained in the digestive tract of carp did not differ between seed sizes, suggesting equal potential dispersal distances for all seeds. Based on optimum swimming speeds of carp, ranging from 0.9 to 1.8 km h<sup>-1</sup>, maximum dispersal distances will most likely range from 13.5 to 27 km.

6. This study highlights the importance of studying all stages of the endozoochorous dispersal process in order to estimate the effect of a phenotypic seed trait on plant dispersal.

*Key-words:* endozoochory, ichthyochory, seed mass, seed size selection, seed traits

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## Introduction

Seed dispersal is assumed to have important fitness advantages for plants, by reducing density-dependent mortality (Escape hypothesis) and increasing the chances of founding a lineage in a new locality (Colonization hypothesis) (Howe & Smallwood 1982), and plays a fundamental role in (meta)population ecology, population genetics and evolutionary biology of plants (Freckleton & Watkinson 2002; Pollux *et al.* 2007).

In tropical regions, internal seed dispersal by fish (endo-ichthyochory) is considered to be an important mode of plant dispersal (Goulding 1980, 1983; De

Souza-Stevaux *et al.* 1994; Kubitzki & Ziburski 1994; Banack *et al.* 2002; Mannheimer *et al.* 2003) that has important consequences for the vegetation along lakes and rivers. Horn (1997), for example, suggested that seed dispersal by the Neotropical characid fish *Brycon guatemalensis* helps maintain an upstream distribution of the riparian fig tree *Ficus glabrata* along river systems of the Costa Rican rain forest, while Gottsberger (1978) argued that the homogeneity of the inundated vegetation along many Amazonian rivers and their tributaries may be caused by the high frequency of fish-mediated seed dispersal. While ichthyochory is also considered to be an important dispersal mechanism in temperate regions (Ridley 1930; Barrat-Segretain 1996), it has hitherto received far less scientific attention (Pollux *et al.* 2006).

The probability of endozoochory for seeds will depend on: (i) the probability that seeds are ingested (Alcántara & Rey 2003; Gómez 2004), which, in turn, is related to both seed availability and the animals' feeding preferences (Jordano 1995; Celis-Diez *et al.* 2004; Bruun & Poschlod 2006); (ii) the time seeds are retained in the digestive system (i.e. retention time), which together with disperser movements affects the potential distance and direction of dispersal (Westcott *et al.* 2005); (iii) the resistance of seeds against digestion, which determines the probability that seeds survive a passage through the intestinal tract of animal dispersers (Charalambidou & Santamaría 2002; Pollux *et al.* 2005); and (iv) the viability and germination rate of seeds after gut passage, which may be decreased, enhanced or unaffected, and determines the probability of germination and successful establishment of the defecated seeds (Traveset 1998; Charalambidou & Santamaría 2002).

Phenotypic seed traits (e.g. seed size, shape, seed coat hardness and the presence of external structure) will have different impacts on each of these four steps in the dispersal process. Both within and between plant species, studies have shown that seed size and seed morphology may negatively impact seed ingestion, yet at the same time, may positively effect seed survival and seed viability, leading to unexpected relationships between phenotypic seed traits and the dispersal and establishment success of seeds (Alcántara & Rey 2003; Gómez 2004; Pollux *et al.* 2006). Thus, when studying the effect of a phenotypic seed trait on the dispersal success of animal-dispersed seeds, it is essential to study all four steps that may affect the probability of seed dispersal (Charalambidou & Santamaría 2002).

Seed size is a phenotypic trait that varies widely within plant species (Michaels *et al.* 1988). When zoochory plays a prominent role in the dispersal of a plant species, differences in probability and distance of dispersal and subsequent success of establishment among differently sized seeds might translate into directional selection pressures on seed size within plant species. Yet, few experimental studies have investigated the effect of intraspecific variation in seed size on the dispersal success and dispersal distance by studying all four steps (Alcántara & Rey 2003; Gómez 2004). In this study we performed a series of controlled feeding experiments to evaluate the effect of seed-size variation within the temperate aquatic macrophyte *Sparganium emersum* (Rehmann 1872, Sparganiaceae) on the probability of dispersal by the common carp (*Cyprinus carpio*, Cyprinidae). We compared the dispersal probabilities of differently sized seeds by assessing the effect of seed size on each of the four aforementioned steps in the process of endozoochorous dispersal. We hypothesized that seed size would have different (potentially counteracting) effects on each of these four dispersal components. Furthermore, we hypothesized that this would translate into differences in dispersal probability and dispersal distance between differently sized seeds.

## Materials and Methods

### EXPERIMENTAL DESIGN

Ripe seeds of *S. emersum* were collected during October 2003 from 75 plants in three natural populations along the River Rur (from Germany to the Netherlands), and stored in a climate chamber (at  $5 \pm 1$  °C) in glass jars filled with tap water. To determine the intraspecific variation in seed mass, the fresh weights of a total of 6463 *S. emersum* seeds were individually measured on a microbalance. Relationships between seed mass and seed size related traits were determined by measuring the mass, length and width of 693 randomly selected *S. emersum* seeds.

Twelve common carp with a mean mass of  $0.307 \pm 0.045$  (SD) kg were obtained from Ruud Vonk Fish Hatchery (Maurik, the Netherlands) in October 2003. The fish were individually kept in 100-L tanks (at 24 °C) in the fish facilities of Radboud University Nijmegen (the Netherlands), and daily fed on a stable diet of commercial pellets (Trouvit, Trouw & Co, Putten, the Netherlands) amounting to 1% of their body mass. To ensure homogenisation of water quality among the twelve tanks, all tanks were supplied with fresh water coming from the same filtering system.

To test the effect of seed mass of *S. emersum* seeds on the probability of their dispersal, three (repeated) feeding trials were performed at weekly intervals (during April and May 2004). In each feeding trial, each of the 12 carp was fed 50 *S. emersum* seeds, though in each trial seeds with a different seed mass: either (L) 'light' seeds (< 10 mg), (M) 'medium' seeds (10–20 mg), or (H) 'heavy' seeds (> 20 mg). The 50 seeds were placed in food pellets (Trouvit pellets soaked with water and formed to pill-shaped balls containing the seeds) before feeding to the carp. The order in which the L, M and H seeds were fed to the carp during the three repeated experiments was partitioned in a randomized complete block design (RCB), with three blocks (block 1: L–H–M; block 2: M–L–H; block 3: H–M–L). Five minutes after feeding, non-ingested seeds (seeds that were expelled by 'spitting'; Sibbing, Osse & Terlouw 1986) were removed from tanks and counted. Next, for a period of 24 h, faeces were collected at 2 h-intervals (preliminary tests, lasting 48 h, showed that the fish always excreted all non-digested seeds well within 24 h). Collected faeces were immediately rinsed with tap water. Retrieved seeds were transferred to plastic containers (100 mL) filled with tap water and returned to the climate chamber for the remainder of the experiments to ensure an equal stratification period for seeds obtained from the three feeding trials (from seed collection in the field in October 2003 to the germination test in May 2004). For each seed mass, three batches of 50 randomly selected non-ingested seeds were used as controls in the germination experiment. These control seeds received a similar pre- and post-experimental treatment as the seeds used in the feeding experiments

to exclude possible effects of pre- or post-feeding treatment of the seeds.

In May 2004, all retrieved and (non-ingested) control seeds were simultaneously set to germinate in a climate chamber with a photoperiod of 16L : 8D, a daytime irradiance of 200  $\mu\text{mol photons s}^{-1} \text{m}^{-2}$  and a day/night temperature cycle of 25/18 °C. Seeds were placed in transparent polystyrene microtiterplates (Omnilabo International BV, the Netherlands), filled with tap water (one seed per well). The morphology of germination of *S. emersum* is similar to that of *S. erectum* (Cook 1962). Germination, in our study defined as the emergence of the first foliage leaf, was checked daily for a period of 45 days.

#### STATISTICAL ANALYSES

Relationships between seed mass and seed size (width and length) were assessed by means of linear regression analysis, using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA).

The probability of seed ingestion (i.e. proportion of offered seeds that were ingested), survival (i.e. proportion of ingested seeds recovered from the faeces) and germination (i.e. proportion of seeds that germinated by the end of the germination run) was assessed by fitting generalized linear models to the data, using the GENMOD procedure in SAS 9.1.3 with a binomial response distribution and a logit link function (SAS Institute Inc., Cary, NC, USA). In the analyses, seed mass and 'block' (of the RCB design) were included as fixed factors. Data from repeated experiments using the same fish individuals were expected to be correlated. To adequately account for this effect and statistically remove the 'nuisance effect' of differences among fish individuals, models were fitted according to the Generalized Estimating Equations (GEE) method (Liang & Zeger 1986) using the REPEATED statement with fish individual treated as the SUBJECT effect and an independent covariance structure (Stokes *et al.* 1995). To evaluate differences between seed masses (L, M and H), pair-wise *post hoc* comparisons of means were performed with a Bonferroni significance level adjustment. In addition, for each seed-mass category the probability of germination of fish-ingested seeds was compared to that of non-ingested (control) seeds, using a  $P < 0.0167$  comparison-wise error rate after Bonferroni correction.

Variation in retention time among seed masses was analysed by fitting General Linear Models, using the MIXED procedure for repeated measures (Littell *et al.* 1998), to seed retrieval rates over time with seed mass, block and retention time as fixed effects and fish as the SUBJECT effect (Charalambidou *et al.* 2003). Prior to the analyses, the data were arcsine (square root) transformed to assure homoscedasticity and normality of residuals. To remove the effect of total survival from this analysis, data were standardized by dividing data from each retrieval event (at each measured retention

time) by the total survival measured in that individual fish (Charalambidou *et al.* 2003).

Differences in germination rates (the number of days to germination) were tested in a survival analysis by fitting a Cox proportional hazards regression to the number of days between setting for germination and seedling emergence for each individual seed that germinated, using S-plus 2000 (Mathsoft Engineering and Education Inc., Zoetermeer, the Netherlands). To separate the effects of germination rate from those of total germination, non-germinated seeds were excluded from the analysis. In addition, we fitted separate models for each seed mass category, comparing the germination rate of fish-ingested vs. control seeds, with seed treatment (fish-ingested vs. controls) as a fixed factor and individual as a random effect.

#### Results

The average (SE) seed mass was 14.09 (0.06) mg ( $N = 6463$  seeds, range = 2.0–35.8 mg, Fig. 1). Seed mass was positively related to seed length (Linear regression:  $R^2 = 0.159$ ;  $P < 0.0001$ ), seed width ( $R^2 = 0.508$ ;  $P < 0.0001$ ) and a multiplication of seed length  $\times$  width ( $R^2 = 0.579$ ;  $P < 0.0001$ ;  $N = 693$  seeds).

The probability of ingestion of *S. emersum* seeds was significantly affected by their seed mass (RMANOVA:  $df = 2$ ,  $\chi^2 = 10.54$ ,  $P = 0.0051$ ), with heavier seeds having a significantly lower probability of being ingested compared to lighter seeds (Fig. 2a; H vs. L:  $df = 1$ ,  $\chi^2 = 58.15$ ,  $P < 0.0001$ ; H vs. M:  $df = 1$ ,  $\chi^2 = 14.15$ ,  $P = 0.0002$ ; M vs. L:  $df = 1$ ,  $\chi^2 = 27.88$ ,  $P < 0.0001$ ). The factor 'block' (of the RCB-design) had no effect on the ingestion of seeds ( $df = 2$ ,  $\chi^2 = 2.40$ ,  $P = 0.3015$ ), indicating that the order in which the seed masses were

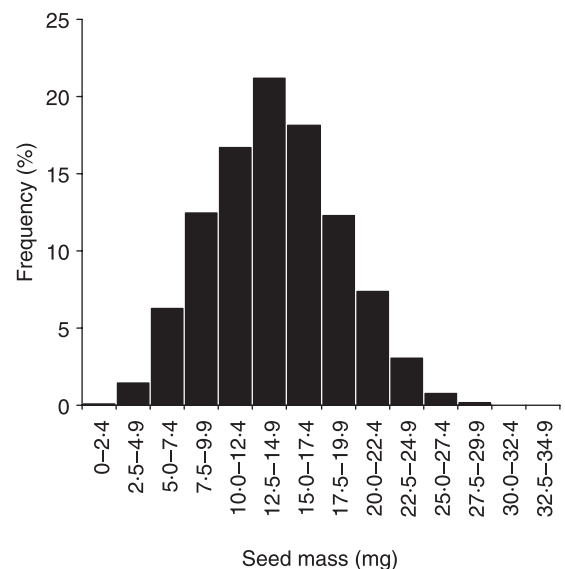
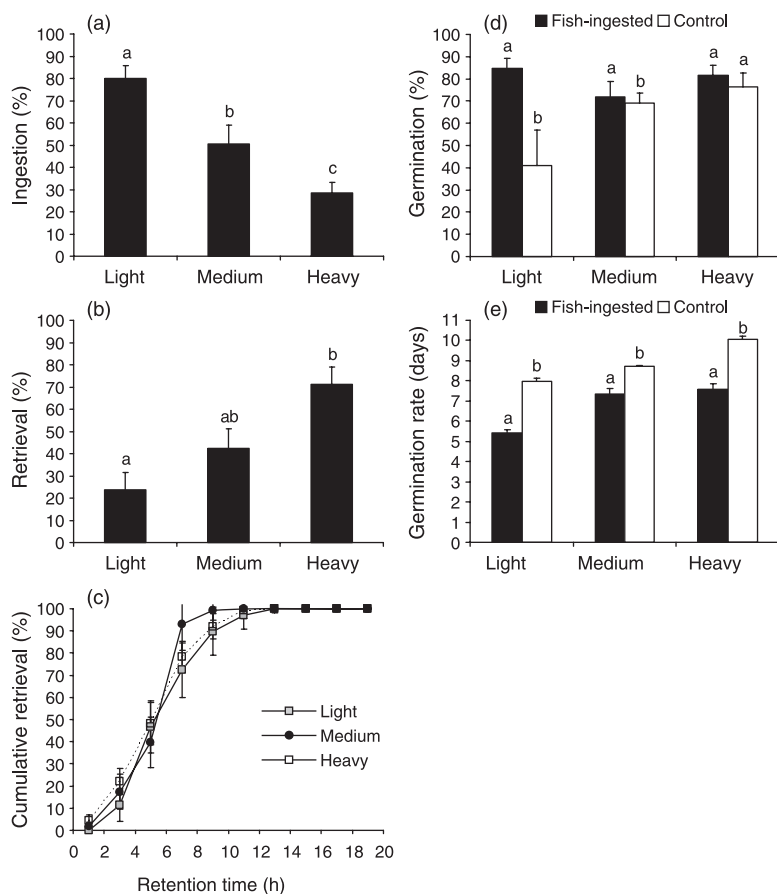


Fig. 1. Distribution of seed mass classes in *Sparganium emersum*, based on 6462 seeds taken from 75 plants collected in three natural populations in the Netherlands.



**Fig. 2.** Mean ( $\pm$  SE) (a) seed ingestion (%), (b) seed survival (%) over 24 h, and (c) cumulative retrieval (%) ([number of seeds retrieved after  $\times$  hours after ingestion/total number of seeds retrieved after 24 h]  $\times$  100) of *Sparganium emersum* seeds fed to carp (bars that do not share a common letter are significantly different from each other; see text for  $P$ -values). Mean ( $\pm$  SE) (d) seed germination (%) and (e) germination rate (number of days to germination) of fish-ingested seeds (black bars) and non-ingested control seeds (white bars;  $n = 150$  controls for each seed mass, in three batches of 50 seeds) of *S. emersum* (adjacent bars that do not share a common letter are significantly different from each other; see text for  $P$ -values). The data presented in a–e is based on  $n = 3$  repeated feeding trials, each trial with  $n = 12$  fish (the order in which each carp was fed the L, M and H seeds in the three feeding trials, was partitioned in a randomized complete block design).

partitioned among the three repeated feeding trials did not affect their probability of ingestion.

The probability of survival of *S. emersum* seeds was also significantly affected by seed mass (d.f. = 2,  $\chi^2 = 6.92$ ,  $P = 0.0314$ ), with heavier seeds having a higher probability of survival compared to lighter seeds (Fig. 2b; H vs. L: d.f. = 1,  $\chi^2 = 15.48$ ,  $P < 0.0001$ ; H vs. M: d.f. = 1,  $\chi^2 = 4.23$ ,  $P = 0.0398^*$ ; M vs. L: d.f. = 1,  $\chi^2 = 3.47$ ,  $P = 0.0625$ ; \*non-significant after Bonferroni correction). The fish faeces contained many seed fragments, especially during the first 10 h, indicating the death of the rest of the ingested seeds. The factor block did not affect the total survival of seeds (d.f. = 2,  $\chi^2 = 1.99$ ,  $P = 0.3697$ ), indicating that the order in which the seed masses were partitioned among the three repeated feeding trials did not affect the probability of seed survival. All seeds were retrieved within 16 h. The pattern of seed retrieval over time

followed a leptokurtic curve, did not differ between the three different seed masses (RMANOVA:  $F_{2,16} = 0.12$ ,  $P = 0.8854$ ; Fig. 2c) and was not affected by the block design ( $F_{2,8} = 0.02$ ,  $P = 0.9757$ ).

The probability of germination after gut passage was neither affected by seed mass (d.f. = 2,  $\chi^2 = 3.78$ ,  $P = 0.1510$ ) nor block effect (d.f. = 2,  $\chi^2 = 1.87$ ,  $P = 0.3935$ ). Separate *post hoc* tests, comparing the germination of fish-ingested vs. control seeds for each seed mass, showed that for light and medium seeds germination of fish-ingested seeds was significantly higher than for control seeds (d.f. = 1,  $\chi^2 = 50.98$ ,  $P < 0.0001$  and d.f. = 1,  $\chi^2 = 22.85$ ,  $P < 0.0001$ , respectively), while for heavy seeds no significant difference was found after Bonferroni adjustment (d.f. = 1,  $\chi^2 = 4.16$ ,  $P = 0.0413$ ; Fig. 2d).

Germination rate (the number of days to germination) of fish-ingested seeds was significantly affected by seed mass (Cox regression: L vs. M:  $\chi^2 = 32.39$ ,  $P < 0.0001$ ; M vs. H:  $\chi^2 = 3.05$ ,  $P < 0.0081$ ), suggesting that light seeds germinated faster than heavier seeds. Separate *post hoc* tests, comparing the germination rate of fish-ingested vs. control seeds, showed that for each seed mass fish-ingested seeds had a higher germination rate (fewer days to germination) than non-ingested control seeds (Cox regression:  $\chi^2 = 16.2$ ,  $P < 0.0001$ ;  $\chi^2 = 11.1$ ,  $P = 0.0009$ ;  $\chi^2 = 5.24$ ,  $P = 0.022$ ; for light, medium and heavy seeds, respectively; Fig. 2e).

## Discussion

### SEED UPTAKE VS. SEED INGESTION

Although some temperate fish species intentionally take up seeds while foraging (García-Berthou 2001; Chick *et al.* 2003), the majority may actually take up seeds unintentionally while foraging on vegetative plant parts or while sifting through the detritus layers on the bottom (Nurminen *et al.* 2003). Whether intentional or unintentional, in waters that are characterized by the presence of large numbers of fish, these may collectively constitute an important factor in the dispersal of aquatic plants (Gottsberger 1978; Horn 1997; Pollux *et al.* 2006).

To mimic the process of unintentional seed uptake by carp, seeds were placed in food pellets before feeding to the carp. Despite the unintentional uptake of seeds into the oral cavity, the proportion of actually ingested (i.e. swallowed) seeds differed significantly between light, medium and heavy seeds (79.8%, 50.4% and 28.6%, respectively). The probability of seed ingestion depends on: (i) selectivity by the animal species; and (ii) the relative availability of seeds (Jordano 1995). In the field, the relative availability of different seed sizes varies for *S. emersum* (Fig. 1). However, in this study we ensured an equal seed availability in all experiments precluding frequency-dependent seed-size selection during ingestion (Celis-Diez *et al.* 2004). The results therefore strongly argue for size selectivity before

actual seed ingestion (swallowing) by carp, despite the unintentional uptake of seeds into the oral cavity.

Fishes have complex mechanical and chemical senses for the examination of potential food items that have been taken up into the oral cavity (Sibbing *et al.* 1986). Here, unpalatable food items (e.g. detritus, sand, stones, hard seeds) are separated from palatable items and subsequently expelled by 'spitting' (Sibbing *et al.* 1986; Callan & Sanderson 2003). Previous experiments have shown that this may lead to differences in seed ingestion rates between plant species with different seed morphologies (Pollux *et al.* 2006). This study shows that ingestion rates also vary intraspecifically, since large *S. emersum* seeds are more likely to be identified as unpalatable items and expelled by carp than small seeds.

#### SEED SURVIVAL AND RETENTION TIME

The proportion of retrieved seeds differed significantly between seed sizes, with smaller seeds having a lower survival percentage compared to larger seeds (23.6%, 42.2% and 71.0%, for light, medium and heavy seeds, respectively). The differences in survival are most likely related to differences in absolute seed coat thickness; that is, larger *S. emersum* seeds have a thicker seed coat compared to smaller seeds, leading to higher survival rates. Seed survival in the digestive tract of vertebrates is known to depend on protection by the seed coat (Traveset 1998), and comparative studies have shown that plant species with hard seed coats have a higher probability of survival than species with soft seed coats when fed to fish (Agami & Waisel 1988; Smits *et al.* 1989; Pollux *et al.* 2006).

The ability to disperse seeds will also vary among fish species depending on their digestive characteristics (Kubitzki & Ziburski 1994). In temperate regions, the common carp is considered to be one of the most efficient seed digesting fish species (Agami & Waisel 1988; Smits *et al.* 1989). While seed survival percentages after ingestion by the common carp differ between plant species, varying from 0% for *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata*; 1%–2% for *Najas marina* and *Ruppia maritima*, 8% for *Potamogeton obtusifolius*; 21% for *Sagittaria sagittifolia*; 29% for *P. pectinatus*; 39% for *S. emersum*; and 67% for *P. natans*, the common carp is still likely to play an important role in the dispersal of several aquatic plant species (Agami & Waisel 1988; Smits *et al.* 1989; Pollux *et al.* 2006).

A study by Horn (1997), showed that *F. glabrata* seeds that are eaten by fish sink to the bottom once defecated, due to removal of the fleshy fruit pulp. In concurrence, we observed that the buoyancy of *S. emersum* seeds was greatly reduced after passing through the digestive tract of carp (in most cases seeds lost their buoyancy entirely). The drupe-like fruit of *S. emersum* consists of a seed enclosed in a hard scleridial endocarp and a tough 'spongy' mesocarp (Cook & Nicholls 1986): The latter, which is filled with numerous

air-chambers (Pollux, unpublished data), was often damaged or even completely removed after gut passage most likely causing the change in buoyancy. These observations indicate that once seeds have been defecated by fish they are likely to sink immediately. Hence, even if fish would not transport seeds they ingested to other locations, they are still likely to affect their potential for hydrochoric dispersal.

The curves of seed retrieval over retention time were identical for all three seed-size categories, despite the large differences in seed weight. These findings are in accordance with other studies that show that in temperate fish species the size, hardness and biochemical composition of various food items (e.g. seeds, invertebrates, prey fish) have little effect on the time required to evacuate the stomach content (Pollux *et al.* 2006; and references therein). In contrast, several studies have shown that in higher vertebrates (e.g. birds, cattle, primates) the size of a seed may determine the time it remains in an animal's digestive tract, often with large and heavy seeds having shorter retention times in the digestive tract of vertebrates than small and light seeds, potentially leading to shorter dispersal distances (Traveset 1998). It is therefore suggested that seed size affects the retention time of seeds passing through the digestive tracts of higher vertebrates with highly specialized digestive systems, but not of seeds passing through the relatively unspecialized digestive tracts of fish.

#### GERMINATION

The proportion of seeds germinating after passing through the digestive tract of carp did not differ between small, medium or large *S. emersum* seeds (proportions ranging 72.8%–84.8%). However, seeds that passed through the digestive tract of carp mostly had a slightly higher germination compared to non-ingested (control) seeds. This increase in germination is most likely related to the breaking of seed-coat dormancy (Baskin & Baskin 1998), which is necessary before germination of *Sparganium* can commence (Cook 1962). Under natural conditions the seed coat of *S. emersum* can be broken by natural decomposition or by a period of freezing. Studies have shown that a passage through the digestive tract of waterfowl or fish may also break seed-coat dormancy of hard-coated plant seeds, due to mechanical and chemical abrasion of the seed coat (Agami & Waisel 1988; Smits *et al.* 1989; Traveset 1998; Santamaría *et al.* 2002; Pollux *et al.* 2005, 2006).

The germination rate was higher (i.e. earlier germination) for smaller compared to larger fish-ingested *S. emersum* seeds, with differences in germination rate ranging from 1 to 3 days. These differences are most likely related to differences in seed coat thickness. During gut passage, the thinner seed coats of small *S. emersum* seeds may have sustained relatively more abrasion than the seed coats of larger seeds, abrasion during gut passage potentially enhancing germination

(Traveset 1998). However, it has been argued, that such small differences in germination rate (1–3 days) between seeds might be too small to be translated into competitive (dis)advantages among seedlings under natural conditions in the field (Figuerola *et al.* 2005; Verdú & Traveset 2005).

#### DISPERSAL PROBABILITY AND DISPERSAL DISTANCE

The probability of seed dispersal [ $p(d)$ ] will depend on the probabilities of: seed availability in the field [ $p(a)$ ], seed ingestion by the animal [ $p(i)$ ], seed survival [ $p(s)$ ] and seed viability after gut passage [ $p(g)$ ]. These probabilities can be inferred from feeding experiments and may subsequently be used to compare dispersal probabilities between different plant species or seed size categories, by calculating the dispersal probability for each plant species or seed-size category as:  $p(d) = p(a) \times p(i) \times p(s) \times p(g)$  (Pollux *et al.* 2006). In our feeding experiments, the availability of the seeds was kept equal for each seed-size category [ $p(a) = 1$ ] to ensure that seed-size selection during ingestion was not frequency-dependent (Celis-Diez *et al.* 2004; Fig. 3a). The results

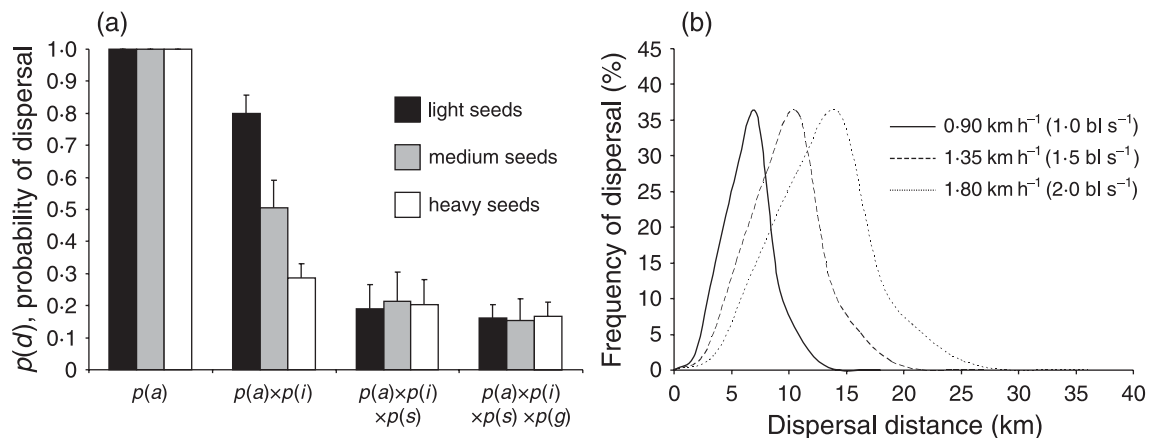
of our study suggest that, although seed size has a pronounced effect on seed ingestion and seed survival, there will be no significant differences in the overall dispersal probability  $p(d)$  of differently sized *S. emersum* seeds. This is because the initial reduction in probability of ingestion  $p(i)$  with increasing seed size (L = 0.798, M = 0.504 and H = 0.286, respectively) is counter-balanced by an increased probability of seed survival  $p(s)$  (L = 0.236, M = 0.422 and H = 0.710), while the probability of germination of retrieved seeds  $p(g)$  remains unaffected by seed size (ranging 0.728–0.848) (Fig. 3a).

The potential dispersal distance of seeds dispersed by carp will depend on: (i) the retrieval rate of seeds passing through the digestive tract of animals (Charalambidou *et al.* 2003); and on (ii) the migratory movements of the animals in the field (Westcott *et al.* 2005). Previous studies have shown that *S. emersum* seeds can stay up to 60 h in the digestive tract of mallard (*Anas platyrhynchos*) and teal (*A. crecca*) (Pollux *et al.* 2005). Assuming linear non-stop migratory movements, and a flying speed for *Anas* ducks that ranges from 60 to 78 km h<sup>-1</sup> (Welham 1994), this would correspond to a maximum dispersal distance of 3600–4680 km (Table 1;

**Table 1.** Comparison of ingestion, survival, germination, retention time (RT) and estimated dispersal distance (assuming non-stop linear migratory movements) of *Sparganium emersum* seeds when fed to waterfowl and fish

Animal vector (mechanism)	Dispersal probability			Dispersal distance				
	Ingestion (%)	Survival (%)	Germination (%)	RT <sub>50%</sub> (h)	RT <sub>90%</sub> (h)	RT <sub>max</sub> (h)	Moving speed (km h <sup>-1</sup> )	Estimated max. distance (km)
Fish* (ichthyochory)	53.97 ± 31.2	45.99 ± 35.1	79.00 ± 19.6	6	8	16¶	0.9–1.8	13.5–27
Waterfowl† (ornithochory)	–‡	22.65 ± 20.8	79.04 ± 19.5	8	44	60§	60–78	3600–4680

\*Common carp (*Cyprinus carpio*), †mallard (*Anas platyrhynchos*) and teal (*Anas crecca*); ‡Ingestion was standardized to 100 seeds per duck; Experiments were terminated at ¶60 and §24 h, respectively (based on this study; Pollux *et al.* 2005; Pollux 2007).



**Fig. 3.** (a) A comparison of the dispersal probability between light (white bars), medium (grey bars) and heavy (black bars) seeds of *Sparganium emersum* dispersed by fish. The comparison is based on parameters inferred from the feeding experiments:  $p(a)$  = probability of seed availability [in this study  $p(a)$  is equal to 1],  $p(i)$  = probability of ingestion,  $p(s)$  probability of survival during gut passage and  $p(g)$  = probability of germination after gut passage. (b) The dispersal curves of *S. emersum* dispersed by fish, based on the seed retention time of *S. emersum* in the intestinal tract of carp (data for light, medium and heavy seeds were pooled as there were no significant differences between them), and three different optimal swimming speeds of carp (ranging 1.0–2.0 bl s<sup>-1</sup>), assuming non-stop linear swimming over a period of 16 h.



Pollux 2007). Common carp inhabiting lowland rivers are also known to display considerable migratory movements, ranging from 5 up to 1000 km (Stuart & Jones 2006). The pattern of seed retrieval by carp shows that carp mediated dispersal is not affected by seed size, and will probably take place over distances within 16 h of swimming, with maximum dispersal probabilities at 4–8 h after ingestion. For the common carp optimum swimming speeds (fish generally swim close to their optimum speed as this is energetically most favourable) is about 1–2 body lengths *per second* ( $\text{bl s}^{-1}$ ) (Ohlberger *et al.* 2006). For the carp used in our experiments, with a fork length of *c.* 25 cm, this would lead to an optimum swimming speed between 0.9 to 1.8  $\text{km h}^{-1}$ , which corresponds to a maximum dispersal distance of 13.5–27 km (at a maximum gut retention time of 16 h; Fig. 3b).

Finally, differences in probability and distance of dispersal and subsequent success of establishment among differently sized seeds may translate into directional selection pressures on seed size within plant species, when zoochory plays an important role in its dispersal. While we have no exact data on the frequency of occurrence of zoochory in *S. emersum*, seed dispersal by fish and waterfowl is considered to play an important role in its dispersal (Pollux *et al.* 2005, 2006; and references therein). However, since we found no differences in either the probability of dispersal, dispersal distance or success of establishment (assessed as rate and percentage of germination) between differently sized seeds when dispersed by fish, ichthyochory is not likely to induce directional selection pressures on seed size within *S. emersum*.

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### References

Agami, M. & Waisel, Y. (1988) The role of fish in distribution and germination of seeds of the submerged macrophytes *Najas marina* L. and *Ruppia maritima* L. *Oecologia*, **76**, 83–88.

Alcántara, J.M. & Rey, P.J. (2003) Conflicting selection pressures on seed size: evolutionary ecology of fruit size in a bird-dispersed tree, *Olea europaea*. *Journal of Evolutionary Biology*, **16**, 1168–1176.

Banack, S. A., Horn, M. H. & Gawlicka, A. (2002) Dispersers vs. establishment-limited distribution of a riparian fig tree (*Ficus insipida*) in a Costa Rican tropical rain forest. *Biotropica*, **34**, 232–243.

Barrat-Segretain, M.H. (1996) Strategies of reproduction, dispersion, and competition in river plants: a review. *Vegetatio*, **123**, 13–37.

Baskin, C.C. & Baskin, J.M. (1998) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego.

Bruun, H.H. & Poschold, P. (2006) Why are small seeds dispersed through animal guts: large numbers or seed size per se? *Oikos*, **113**, 402–411.

Callan, W.T. & Sanderson, S.L. (2003) Feeding mechanisms in carp: crossflow filtration, palatal protrusions and flow reversals. *Journal of Experimental Biology*, **206**, 883–892.

Celis-Diez, J.L., Bustamente, R.O. & Vásquez, R.A. (2004) Assessing frequency-dependent seed size selection: a field experiment. *Biological Journal of the Linnean Society*, **81**, 307–312.

Charalambidou, I. & Santamaría, L. (2002) Waterbirds as endozoochorous dispersers of aquatic organisms: a review of experimental evidence. *Acta Oecologica*, **23**, 165–176.

Charalambidou, I., Santamaría, L. & Langevoord, O. (2003) Effect of ingestion by five avian dispersers on the retention time, retrieval and germination of *Ruppia maritima* seeds. *Functional Ecology*, **17**, 747–753.

Chick, J.H., Cosgriff, R.J. & Gittinger, L.S. (2003) Fish as potential dispersal agents for floodplain plants: first evidence in North America. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**, 1437–1439.

Cook, C.D.K. & Nicholls, M.S. (1986) A monographic study of the genus *Sparganium* (Sparganiaceae). Part I. Subgenus *Xanthosparganium* Holmberg. *Botanica Helvetica*, **96**, 213–267.

Cook, C.D.K. (1962) *Sparganium erectum* L. (*S. ramosum* Hudson, *nom. illeg.*). *Journal of Ecology*, **50**, 247–255.

De Souza-Stevaux, M. C., Negrelle, R.R.B. & Citadini-Zanette, V. (1994) Seed dispersal by the fish *Pterodoras granulosus* in the Parana River basin, Brazil. *Journal of Tropical Ecology*, **10**, 621–626.

Figuerola, J., Santamaría, L., Green, A., Luque, I., Alvarez, R. & Charalambidou, I. (2005) Endozoochorous dispersal of aquatic plants: does seed gut passage affect plant performance? *American Journal of Botany*, **92**, 696–699.

Freckleton, R.P. & Watkinson, A.R. (2002) Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. *Journal of Ecology*, **90**, 419–434.

García-Berthou, E. (2001) Size- and depth-dependent variation in habitat and diet of the common carp (*Cyprinus carpio*). *Aquatic Sciences*, **63**, 466–476.

Gómez, J.M. (2004) Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution*, **58**, 71–80.

Gottsberger, G. (1978) Seed dispersal by fish in the inundated regions of Humaita, Amazonia. *Biotropica*, **10**, 170–183.

Goulding, M. (1980) *The fishes and the forest: explorations in Amazonian natural history*. University of California Press, Berkeley.

Goulding, M. (1983) The role of fishes in seed dispersal and plant distribution in Amazonian floodplain ecosystems. *Sonderbände des Naturwissenschaftlichen Vereins in Hamburg*, **7**, 271–283.

Horn, M.H. (1997) Evidence for dispersal of fig seeds by the fruit-eating characid fish *Brycon guatemalensis* Regan in a Costa Rican tropical rain forest. *Oecologia*, **109**, 259–264.

Howe, H.F. & Smallwood, J. (1982) Ecology of seed dispersal. *Annual Review of Ecology and Systematics*, **13**, 201–228.

Jordano, P. (1995) Frugivore-mediated selection on fruit and seed size: birds and St. Lucie's cherry, *Prunus mahaleb*. *Ecology*, **76**, 2627–2639.

Kubitzki, K. & Ziburski, A. (1994) Seed dispersal in flood plain forests of Amazonia. *Biotropica*, **26**, 30–43.

Liang, K.Y. & Zeger, S.L. (1986) Longitudinal data analysis using generalized linear models. *Biometrika*, **73**, 13–22.

Littell, R.C., Henry, P.R. & Ammerman, C.B. (1998) Statistical analysis of Repeated Measures data using SAS Procedures. *Journal of Animal Science*, **76**, 1216–1231.

- Mannheimer, S., Bevilacqua, G., Caramaschi, E.P. & Scarano, F.R. (2003) Evidence for seed dispersal by the catfish *Auchenipterichthys longimanus* in an Amazonian lake. *Journal of Tropical Ecology*, **19**, 215–218.
- Michaels, H.J., Benner, B., Hartgerink, A.P., Lee, T.D., Rice, S., Willson, M.F. & Bertin, R.I. (1988) Seed size variation: magnitude, distribution, and ecological correlates. *Evolutionary Ecology*, **2**, 157–166.
- Nurminen, L., Horppila, J., Lappalainen, J. & Malinen, T. (2003) Implications of rudd (*Scardinius erythrophthalmus*) herbivory on submerged macrophytes in a shallow eutrophic lake. *Hydrobiologia*, **506–509**, 511–518.
- Ohlberger, J., Staaks, G. & Hölker, F. (2006) Swimming efficiency and the influence of morphology on swimming costs in fishes. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, **176**, 17–25.
- Pollux, B.J.A. (2007) *Plant dispersal in rivers – A mechanistic and molecular approach*. PhD thesis, Radboud University Nijmegen, Nijmegen.
- Pollux, B.J.A., De Jong, M., Steegh, A., Ouborg, N.J., Van Groenendael, J.M. & Klaassen, M. (2006) The effect of seed morphology on the dispersal of aquatic macrophytes by the common carp (*Cyprinus carpio*). *Freshwater Biology*, **51**, 2063–2071.
- Pollux, B.J.A., De Jong, M., Steegh, A., Verbruggen, E., Van Groenendael, J.M. & Ouborg, N.J. (2007) Reproductive strategy, clonal structure and genetic diversity in populations of the aquatic macrophyte *Sparganium emersum* in river systems. *Molecular Ecology*, **16**, 313–325.
- Pollux, B.J.A., Santamaria, L. & Ouborg, N.J. (2005) Differences in endozoochorous dispersal between aquatic plant species, with reference to plant population persistence in rivers. *Freshwater Biology*, **50**, 232–242.
- Ridley, H.N. (1930) *The Dispersal of Plants Throughout the World*. Reeve & Co. Ltd, Ashford, Kent.
- Santamaria, L., Charalambidou, I., Figuerola, J. & Green, A.J. (2002) Effect of passage through duck gut on germination of fennel pondweed seeds. *Archiv für Hydrobiologie*, **156**, 11–22.
- Sibbing, F.A., Osse, J.W.M. & Terlouw, A. (1986) Food handling in the carp (*Cyprinus carpio*): its movement patterns, mechanisms and limitation. *Journal of Zoology Series A*, **210**, 161–203.
- Smits, A.J.M., Van Ruremonde, R. & Van der Velde, G. (1989) Seed dispersal of three Nymphaeid macrophytes. *Aquatic Botany*, **35**, 167–180.
- Stokes, M.E., Davis, C.S. & Koch, G.G. (1995) *Categorical Data Analysis Using the SAS System*. SAS Institute, Cary, North Carolina, USA.
- Stuart, I.G. & Jones, M.J. (2006) Movement of common carp, *Cyprinus carpio*, in a regulated lowland Australian river: implications for management. *Fisheries Management and Ecology*, **13**, 213–219.
- Traveset, A. (1998) Effect of seed passage through vertebrate frugivores's guts on germination: a review. *Perspectives in Plant Ecology, Evolution and Systematics*, **1/2**, 151–190.
- Verdú, M. & Traveset, A. (2005) Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. *Ecology*, **86**, 1385–1394.
- Welham, C.V.J. (1994) Flight speeds of migrating birds: a test of maximum range speed predictions from the aerodynamic equations. *Behavioral Ecology*, **5**, 1–8.
- Westcott, D.A., Bentrupperbäumer, J., Bradford, M.G. & McKeown, A. (2005) Incorporating patterns of disperser behaviour into models of seed dispersal and its effects on estimated dispersal curves. *Oecologia*, **146**, 57–67.

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