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Locomotion during digestion changes current estimates of seed dispersal kernels by fish

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Summary

1. Dispersal of seeds by animals is an important mechanism regulating plant diversity, range expansions and invasions. Many birds, mammals, fish and reptiles regularly ingest, transport and excrete viable seeds (known as endozoochory).

2. The effectiveness of endozoochory is modelled in dispersal kernels: functions that describe seed shadows in the landscape by combining movement of animals with experimentally obtained seed retention times and survival.

3. Currently, dispersal kernels use experimental data from resting animals, yet only moving animals disperse seeds. Although physical activity is known to affect digestive processes, little is known on how and to what extent this may influence current estimates of endozoochory. Activity may either prolong seed retention in the animal's gut (locomotion-priority mode hypothesis) or may not affect seed excretion rate (digestion-priority mode hypothesis), and may affect seed survival and germination positively or negatively.

4. We tested how activity alters dispersal estimates in fish. We compared the seed dispersal potential of two riparian plant species (*Carex acuta* and *Carex riparia*) by the common carp (*Cyprinus carpio*) subjected to three different activity levels: low (basal metabolic rate, BMR), medium (2 × BMR) or high activity (3 × BMR).

5. Physical activity of the fish did not affect the number of intact retrieved seeds over 15 h of activity, but significantly affected seed retrieval patterns over time for both seed species. More active fish started seed excretion about 1 h later and kept excreting seeds at least 2 h longer. Effects of gut passage on germination could only be tested for *C. acuta*, where it reduced the percentage of germinating seeds by 22%, independent of the activity level. Seeds ingested by the fish germinated on average 3·5 days later than non-ingested control seeds. Seed retention times did not affect the timing of germination.

6. Our results support the locomotion-priority mode hypothesis and show that modelling dispersal kernels using parameters from inactive fish may underestimate potential dispersal distances. Because a trade-off between physical activity and digestive physiology is likely common in animals, it should be taken into account in future modelling of endozoochorous seed dispersal kernels.

Key-words: *Carex*, *Cyprinus carpio*, endozoochory, germination, ichthyochory, metabolic rate, riparian plants, seed retention time

Introduction

Long-distance dispersal is an essential process in shaping species distributions and therefore global biodiversity (Clobert *et al.* 2001; Bullock, Kenward & Hails 2002). Biodiversity at any given location depends on many factors, but an essential primary regulator is the selection of species that is able to reach this location from elsewhere (Dieckmann, O'Hara & Weisser 1999; Van Leeuwen *et al.*

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2014). A lack in seed mobility has been identified as one of the main causes of the current decline of plant species richness following habitat fragmentation in, for instance, north-west Europe (Ozinga *et al.* 2009; Brederveld *et al.* 2011). Similarly, range expansions and plant invasions also depend largely on effective seed dispersal (Kokko & Lopez-Sepulcre 2006).

Seed transport by animals is an effective long-distance dispersal mechanism for many plants. A wide variety of animals, ranging from donkeys to turtles to slugs (Couvreur *et al.* 2005; Sumoski & Orth 2012; Boch *et al.* 2013), and from fish to boars to birds (Herrera *et al.* 1994; Horn *et al.* 2011; Dovrat, Perevolotsky & Ne'eman 2012; Van Leeuwen *et al.* 2012), forage on plant seeds or other dispersible propagules, carry them along and excrete them elsewhere (endozoochory). The effectiveness of seed dispersal by endozoochory is universally estimated by combining data on animal movement with experimental data on seed retention and seed survival in the digestive system of animals (Cousens *et al.* 2010). This combination allows the construction of 'dispersal kernels': models that describe potential seed shadows from the location of ingestion (e.g. Anderson *et al.* 2011; Viana *et al.* 2012; Bialozyt *et al.* 2014).

Models of seed shadows based on dispersal kernels rely on data of seed retention in the vectors, and seed survival and germinability after transport (see a recent review on these models by Cousens *et al.* 2010). However, such data is lacking for many systems, impeding advancement in the field of zoochory (Bauer & Hoye 2014). One particular issue is that all studies to date model dispersal kernels using experimental data on seed retention and seed survival obtained from animals in rest, while only moving animals can contribute to long-distance seed dispersal. Dispersal kernels combine data on animal movement speeds with seed retention times in the digestive tract. However, here we hypothesize that the physical activity required for movement will significantly alter digestive processes and therewith modify the predicted seed shadows.

Locomotion and digestion impose competing metabolic demands on animals, because both muscular work for locomotion and digestive nutrient assimilation lead to an increased oxygen demand (Jobling 1983; Farrell *et al.* 2001). A trade-off between locomotion and digestion could result in either less efficient processing of food during locomotion (locomotion-priority mode) or a decreased movement performance while digesting food (digestion-priority mode) (Palstra & Planas 2013). When faced with this dual demand, some animals prioritize blood flow from the gastrointestinal system to the axial muscles, which delays gut passage rates of food (McGaw 2007; Secor & White 2010). However, in other animals, digestion remains prioritized during locomotion. In these situations, digestive processes should not be reduced and food passage rates are expected to remain the same. Therefore, there are two contrasting hypotheses on how physical activity in animals may alter dispersal kernels: Activity may either delay seed excretion (locomotion-priority mode hypothesis) or may have no effect on seed excretion rate (digestion-priority mode hypothesis, see Fig. 1). Still little is known on how this trade-off between digestion and locomotion may affect endozoochory and subsequently the projected dispersal distance predicted by modelling dispersals kernels.

Here, we contrast the locomotion- and digestion-priority mode hypotheses (Fig. 1) using fish, dispersing seeds of wetland plants, as a model system. Fish are important seed vectors in wetlands and riverine systems (Horn *et al.* 2011; Pollux 2011). In unidirectional landscapes such as river systems, long-distance dispersal is especially essential (Pollux *et al.* 2009a; Honnay *et al.* 2010): As most plants are sessile, their propagules (ranging from sexual seeds to clonal tubers, turions, or plant fragments) are primarily transported downstream by water (hydrochory, Pollux *et al.* 2009b; Nilsson *et al.* 2010; Palstra & Planas 2013). Because only upstream dispersal can counteract stochastic extinction events at higher sections of a river, and thereby ensure long-term plant (meta-)population persistence, organisms in such systems are especially dependent on biotic mechanisms for upstream-directed dispersal ('The Drift Paradox', Speirs & Gurney 2001; Pollux, Santamaría & Ouborg 2005; Mari *et al.* 2014). Animals such as mammals and birds are increasingly identified as vectors playing this role (Van Leeuwen *et al.* 2012), but recent work specifically

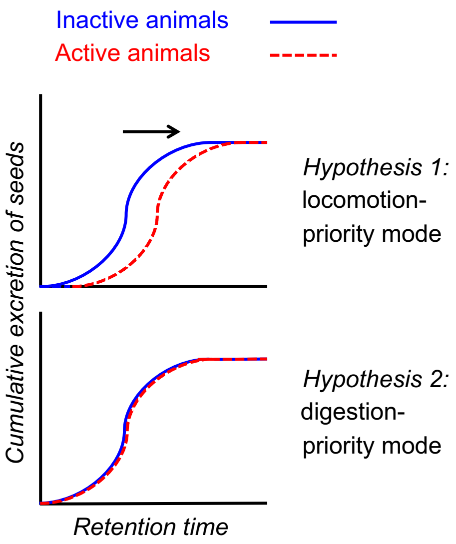

Fig. 1. Current seed dispersal distances are estimated based on experiments with inactive animals in captivity. The time between ingestion and excretion of a seed (on the horizontal axes) correlates positively to seed dispersal distance. Two contrasting hypotheses exist that propose how physical activity of animals can affect seed dispersal distances via modifications of the digestive system. Compared to inactive animals (solid lines), retention times of active animals (dashed lines) can increase due to a reduction of blood flow to their digestive system during physical activity (locomotion-priority mode). Alternatively, digestion can remain prioritized during locomotion, in which case no effects on seed retention times are expected (digestion-priority mode). We test these contrasting hypotheses in fish.

highlights the underestimated potential role of fish (Horn *et al.* 2011).

There is currently no empirical knowledge on how physical activity affects seed retention time and probability of seed digestion in fish, and as a result, all dispersal models are still based solely on experiments with fish in rest (see review by Pollux 2011). Here, we test whether, and how, physical activity of fish modifies their role as dispersal vectors for wetland plants through changes in retention time and/or survival probability of seeds during gut passage. Our model species is common carp (*Cyprinus carpio* L.), one of the most studied fish species in the world and an important seed disperser in temperate regions (Horn *et al.* 2011).

Materials and methods

FISH SPECIES

The experiments were carried out with common carp, a widely distributed freshwater fish that behaves well in laboratory experiments (e.g. Pollux *et al.* 2006). Cypriniformes (carp and minnows) are the most important group of seed dispersers in temperate regions (Horn *et al.* 2011). In their natural habitat, carps are omnivores that feed opportunistically on detritus, zooplankton, insects, crustaceans, molluscs, algae and aquatic plants (García-Berthou 2001; Sinclair *et al.* 2014). Fruits and seeds also constitute an important part of the diet of carp (Crivelli 1981; García-Berthou 2001; Horn *et al.* 2011).

We obtained eight 2-year-old sexually mature carp with a mean body mass of 535·93 g (±73·52 SD) and fork length of 277 mm (±17 SD) from the Aquatic Research Facilities of Wageningen University and Research Centre, the Netherlands (ARF-WUR). These fish are highly similar to fish previously used in detailed published metabolic experiments (Ohlberger *et al.* 2005), enabling accurate estimates of metabolic rates. All fish were individually kept in aquaria (LWH: 0·60 × 0·40 × 0·40 m) placed side by side. Visual contact with neighbours was possible at all times. The aquaria were filled with groundwater (up to 0·35 m), and the water was subsequently purified by means of biological filters. The pH was stabilized between 6·8 and 7·5, ammonium levels were kept <5 mg L^{-1}, and nitrite and nitrate <0·5 mg L^{-1}. Water temperature was regulated to 21 ± 1·0 °C by controlling the room temperature.

FLUME TANK

In the same room, we constructed an oval-shaped flume tank of PVC (Fig. S1, Supporting information). The water in the flume tank was continuously purified by means of a large (100 L) biological filter containing active colonies of water-purifying bacteria. In the flume tank, two silent outboard engines on 12-V batteries were positioned at the start of either long side of the oval, each producing a maximum of 214-N thrust to create controllable flows of different current speeds. At the end of each long side, two rectangular cages (LWH: 0·72 × 0·46 × 0·10 m with a mesh size of 12 mm) kept the fish in their designated individual swim sections. Fish faeces were collected in nylon filters with 0·680-mm-diameter mesh downstream of the cages. Fish that shared one sieve were fed different seed species, which allowed four fish to be in the flume tank simultaneously. The speed of the water current was determined with a Valestrom water current measurer at the positions in the tank that the fish preferred for swimming (i.e. the lowest possible current in the cross-sectional flow profile).

PRE-EXPERIMENTAL TRAINING PERIOD

Starting in May 2013, we trained all fish twice per week for 10 weeks to swim in the flume tank. Maximum sustainable swim speeds were first determined in pilot experiments (results not shown) and corresponded with previously published literature (carp of 262 mm average body length could swim steadily at 0·45 m s^{-1}, Tudorache *et al.* 2007). At the end of the training period, all fish were able to swim continuously for 15 h at a flow velocity of 0·4 m s^{-1}. This speed is within the aerobic scope of the animals (Johnston, Davison & Goldspink 1977; Rome, Loughna & Goldspink 1984). The fish were further trained to voluntarily ingest Trouvit food pellets (Trouw & Co, Putten, the Netherlands) with a diameter of 10 mm containing a fixed number of seeds.

EXPERIMENTAL DESIGN

In the experiment, we subjected the fish to three different treatments: *low-active fish* were placed in the flume tank for 15 h without a water current, *medium-active fish* were swimming at 0·25 m s^{-1} (~1 body length s^{-1}), and *highly active fish* were swimming at 0·40 m s^{-1} (~1·5 body lengths s^{-1}), for 15 h in all treatments. The basal metabolic rate (BMR) of our carp was likely around 60 mg O_2 h^{-1} (as calculated based on the experiments by Huntingford *et al.* 2010). Swimming increased the BMR with an active metabolic rate (AMR) component. Experimentally estimated minimum AMR of highly similar common carp (originating from the same breeding facilities at Wageningen University) follow the function $0{\cdot}021 \times M^{0{\cdot}8} \times U^{0{\cdot}95}$, with M being body mass (g) and U being the absolute swimming speed (cm s^{-1}) (Ohlberger *et al.* 2005). Given the average body mass (536 g) and swimming speeds in our experiment, the estimated total metabolic rates (BMR + AMR) of the active carp were 118 mg O^2 h^{-1} for medium and 166 mg O^2 h^{-1} for highly active individuals. We therefore estimate the carp′s metabolism increased roughly two-fold during the medium and three-fold during the high-activity treatments.

To enable a comparison between two seed species (*Carex acuta* and *Carex riparia*), the eight individual fish underwent each treatment twice: once after ingesting *C. acuta* and once after ingesting *C. riparia,* in a randomized block design with four fish per day over 12 days (1st–12th of August, 2013). Each fish was assigned to the same cage during the six times in the flume tank, thus making sure the only factor that changed between treatments was the current speed and the experimental day. This way, possible variation between cages could be included jointly with individual variability in the statistical models.

At 8:00 AM on each experimental day, four fish were each fed 100 seeds within their own aquarium, using five food pellets per fish containing 20 seeds per pellet. Two fish were fed seeds of *C. acuta,* and two fish were fed seeds of *C. riparia.* The seeds were obtained from Biodivers B.V. (Oudewater, the Netherlands) who collected them from natural populations in the Netherlands <1 year previously. Prior to the feeding, the biological filters were temporarily deactivated to prevent seeds from entering the pumps. The moment that all food pellets were ingested was assigned $t = 0$, at which time the fish were transported to the flume tank. Non-ingested seeds in the aquaria were collected and counted. The transport of fish to the flume tank probably temporarily elevated stress levels. To control for a potential ‘stress effect’ on the seed retrieval patterns, all fish (low, medium and highly active) received the same treatment. After a 15-min acclimation period at zero flow, the trials started by increasing the flow velocity to the designated speed for that day (either 0, 0·25 or 0·40 m s^{-1}).

During the 15-h trials, seeds were collected from the sieves every hour and counted. We hereafter refer to these as ‘defaecated’ seeds. In the treatment without flow, the engines were turned on for 2 min every hour at slow speed to move faeces into the sieves. We assumed that seeds retrieved in the first 4 h after feeding (a total of 45 seeds, <2·3% of the total number retrieved) were expelled by 'spitting' (thus without passing through the digestive system) and we added these to the non-ingested seeds (Sibbing, Osse & Terlouw 1986; Pollux *et al.* 2006). After 15 h, we returned the fish to their individual aquaria. At $t = 24$ h, these aquaria were checked once more for the presence of defaecated seeds. As only four fish were used simultaneously on a single day, each fish had 1 day of rest between experimental days. Fish were fed *ad libitum* with Trouvit food pellets directly after the 15-h experiment.

At the end of the experiment, we euthanized the fish by overdosing with MS222, after which their body weight and fork length were measured. Seed weights of *C. acuta* and *C. riparia* were determined by weighing 20 batches of 20 seeds on a Sartorius scale (with $d = 0{\cdot}001$ g), and three morphological traits were characterized: seed length, width and height were determined to the nearest 0·01 mm using digital callipers.

SEED GERMINATION

Defaecated seeds were rinsed with tap water and transferred to 1·5-mL polypropylene tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) filled with tap water. As control seeds, 10 batches of 50 randomly selected seeds per plant species were directly stored in the same way. All seeds were placed in a dark cold room (7 °C) for 5 months to allow cold stratification prior to the germination experiment.

On 14th January 2014, all control and experimental seeds were set to germinate simultaneously in a climate chamber (Sanyo growth Cabinet; Sanyo Gallenkamp BV, Ettenleur, the Netherlands) with a photoperiod of 16-h light/8-h dark, a daytime irradiance of 60 μmol s^{-1} m^{-2} and a day/night temperature cycle of 24/18 °C. Seeds were placed on Whatman™ filter paper circles (GE Healthcare Life Sciences, Buckinghamshire, UK) in transparent polystyrene Petri dishes (diameter: 90 mm) filled with 5 mL tap water. Germination, defined as the emergence of the first root or foliage leaf, was checked three times per week for a period of 6 weeks. Seeds of *C. riparia* unfortunately did not germinate (probably the seeds already died prior to purchase), which is why we only present the germination results for *C. acuta*.

DATA ANALYSES

Differences between the species in seed morphology were determined using MANOVA, after assessing normality and homoscedasticity of variances. Seed length, height, width and mass depended on the factor seed species. Seeds of *C. riparia* were significantly larger in length and height than seeds of *C. acuta*, but the species did not differ in seed width or mass (Table S1).

Effects of carp activity and plant species on seed ingestion and retrieval were analysed in multiple generalized linear mixed-models (GLMMs). Model selection was based on AICc values (for small sample sizes) and was performed by backward removal of explanatory predictor variables and their interactions starting from full models including all second-order interaction terms (see Table S2). During the model selection procedure, we considered models with AICc values differing in 2·0 or less as equal, and in these cases, we selected the simplest model by the principle of parsimony (Burnham & Anderson 2002; Burnham, Anderson & Huyvaert 2011). GLMM 1 and 2 were fitted with binomial denominators with logit link functions to model the proportions, GLMM 3 was fitted with a Gaussian distribution with log link function, and GLMM4a and 4b were fitted with a Poisson distribution with log link function. Experimental day (12 levels) and carp individual (8 levels) were included in all models as random factors (individual includes variation due to swim cage as each individual was always assigned to the same cage). In GLMM 4a and 4b, additive overdispersion was modelled by adding an extra random factor with a random level for each observation according to Nakagawa & Schielzeth (2010), to absorb overdispersion. The covariate retention time was centred by subtracting the mean from all values (following Raudenbush & Bryk 2002).

Because the carp did not ingest all 100 seeds upon feeding, we first compared potential differences in the number of ingested seeds between plant species. In GLMM1, the proportion of seeds ingested was the dependent variable with seed species as fixed factor.

In GLMM2, we tested whether the total proportion of excreted seeds after 15 h (the active period) differed between the activity levels. The proportion of retrieved seeds was dependent on the fixed factor activity level (3 levels: low, medium and high activity), plant species (2 levels: *C. acuta* and *C. riparia*) and their interaction.

In GLMM3, we analysed the effects of plant species and activity level on the temporal patterns of seed excretion. The central value of the hourly seed retention times of individual seeds was the dependent variable with plant species, fish activity level and their interaction as terms of interest.

To illustrate the differences between dispersal kernels based on experimental data for active and inactive carp, we constructed two simple dispersal kernels based on obtained retention times and hypothetical movement data of carp. Potential dispersal distances of plant seeds (data for *C. acuta and C. riparia* were pooled) were calculated based on our experimental data from active carp (data from medium and high activity were pooled) and inactive carp. The kernels were constructed by multiplying the central value of the hourly seed retention times with the estimated distance covered by a carp of average length (27·7 cm) swimming at 1·0 body length s^{-1} (estimated to be realistic movements based on radiotelemetry monitoring, see Discussion).

Total germination percentage was compared between activity levels using two mixed-effects ANOVAS. In the first model, the percentage of seeds that had germinated after 41 days depended on the fixed factor treatment and random factor individual. In the second similar ANOVA, control seeds were not included, but the interaction of (centred) retention time with treatment was added.

Differences in germination rate were compared between activity levels in GLMM4a and 4b. In model 4a, the days until seeds germinated depended on treatment as fixed factor (with low, medium, high activity or control seeds as levels). In the similar model 4b, the days until seeds germinated depended on treatment as fixed factor without the control seeds included, which allowed testing for effects of the covariate retention time and a potential treatment × retention time interaction, with individual and day included as random factors. Tukey HSD *post hoc* tests with Bonferroni corrections were used to detect differences between levels of the fixed factor treatment. All statistics were performed in R for statistics (R-Development-Core-Team 2015).

Results

SEED INGESTION

The probability of seed ingestion by pellets differed significantly between the plant species (effect of seed species in GLMM1, see Table 1). Although all seeds were initially ingested in the pellets, spitting occurred more often in *C. riparia* seeds. Fish therefore ultimately ingested more seeds of the smaller *C. acuta* (53·9 ± 15·9% SD) than of *C. riparia* (35·7 ± 16·2% SD).

Table 1. (a, b) Results from mixed models exploring factors influencing seed retention, survival and germination by common carp during different levels of activity. Model selection was based on the change of AICc values due to removal of terms (see Table S2 for the model selection procedure). (a) Results of likelihood ratio tests between models including and excluding terms. Bold terms remained in the best models after model selection. (b) Parameter estimates and their significance for significant terms included in the final models. For the factor seed species, *Carex acuta* was set as intercept, and results of GLMM 4a and 4b are for *C. acuta* only

(a)

GLMM	Dependent variable	Term	X^2	d.f.	*P*-value
1	Proportion seeds ingested	**Plant species**	2477·4	1	<0·001
2	Proportion of retrieved seeds	Plant species	0·0023	1	0·96
		Activity level	0·19	1	0·91
		Activity level × plant species	0·53	2	0·77
3	Retention time retrieved seeds	**Plant species**	0·74	1	0·39
		Activity level	3·7	2	0·16
		Activity level × plant species	19·3	2	<0·001
4a	Days until germination	**Treatment**	23·8	3	<0·001
4b	Days until germination	Activity level	1·45	2	0·48
		Retention time	0·01	1	0·93
		Activity level × retention time	0·26	2	0·88

(b)

GLMM	Dependent variable	Explanatory predictor variable	Contrasts	Estimate	SE	*Z*	*t*	*P*-value
1	Proportion seeds ingested	Intercept		−0·16	0·15	−1·05		0·29
		Plant species		0·78	0·016	50·00		<0·001
3	Retention time retrieved seeds	Intercept		2·31	0·046		49·7	<0·001
		Plant species		−0·02	0·016		−1·11	0·26
		Activity level	Low–medium	0·13	0·061		2·05	<0·05
			Low–high	0·02	0·061		0·37	0·71
			Medium–high	−0·10	0·058		−1·68	0·092
		Plant species × activity level	Low–medium	0·01	0·021		0·26	0·79
			Low–high	0·08	0·023		3·67	<0·001
			Medium–high	0·08	0·019		3·97	<0·001
4a	Days until germination	Treatment	Low–medium	−0·41	0·21	−1·94		0·21
			Low–high	−0·20	0·20	−1·00		0·75
			Medium–high	0·21	0·20	1·02		0·73
			Control–low	−1·39	0·28	−5·01		<0·001
			Control–medium	−1·79	0·28	−6·36		<0·001
			Control–high	−1·59	0·27	−5·80		<0·001

SEED RETRIEVAL

The proportion of ingested seeds that was excreted after 15 h was similar between the different activity levels (overall mean proportion for low-activity fish: 0·88 ± 0·086 SD, for medium-activity 0·91 ± 0·083 SD and for high-activity levels 0·89 ± 0·11 SD). There was no significant difference for either of the two plant species (GLMM2 in Table 1). However, the retrieval time course for active fish was not plateaued after 15 h, especially not for *C. riparia* (Fig. 2b), while for the low-activity fish, they seemed to already reach a plateau. The additional proportion of seeds retrieved in the interval between 15 and 24 h, after activity was terminated, was low in all activity levels (low: one fish excreted three seeds, 0·0054 ± 0·021 SD, medium: four fish excreted six seeds, 0·0084 ± 0·017 SD and high: four fish excreted eight seeds, 0·014 ± 0·031 SD).

RETENTION TIME

The temporal patterns of seed excretion changed with the activity levels of the fish. Excretion of seeds by low-activity fish started after 8 h and already saturated 12 h after feeding (Fig. 2), while for active fish, excretion only started to increase 9 h after ingestion and lasted until at least 15 h after ingestion. However, the effects of activity on seed retention times were species-specific (significant interaction of activity level with plant species in GLMM3, Table 1). For both plant species, seed retention times were longer in the medium-activity than in low-activity treatment (Fig. 2). However, a further increase of swimming speed to the high-activity level did not further increase retention times for either plant species (Figs S2 and S3). In *C. acuta,* excretion patterns from high-activity fish did not differ from the excretion patterns by low-activity fish, while in *C. riparia*, retrieval patterns did not differ significantly between medium- and high-activity treatments (Table 1, Fig. 2b, Fig. S3). Detailed differences in retention times between fish at different activity levels are further visualized in the Fig. S2.

To illustrate how these activity-induced changes in seed excretion patterns will likely affect seed dispersal kernels, we constructed Fig. 3 (see Materials and methods). We therein combined hypothetical movement data from

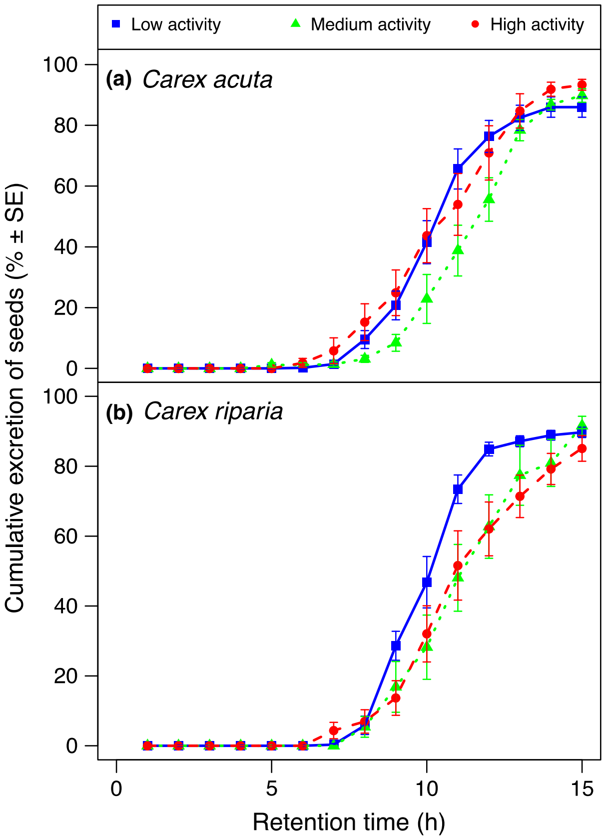

Fig. 2. Cumulative retrieval of (a) *Carex acuta* and (b) *Carex riparia*, with the number of seeds ingested assumed as 100% of possible retrieval. The retrieval is indicated for the three activity levels. Note that the error bars are standard errors for clarity.

common carp with average retention time data from either all active or all inactive fish (pooled for both seed species). The median of the seed excretion differed significantly between the two constructed kernels (Welch's two-sample *t*-test between the median moment of seed retrieval from active and inactive fish: 10·8 h ± 1·6 SD and 9·9 h ± 0·7 SD, respectively, $t = -2{\cdot}75$, d.f. = 44·8, $P = 0{\cdot}0085$). The tail of the dispersal kernel (described as the upper quartile of the moment of seed retrieval) was also significantly fatter in active fish than in inactive fish (Welch's two-sample *t*-test: 11·6 h ± 1·5 SD and 10·7 h ± 0·8 SD, respectively, $t = -2{\cdot}74$, d.f. = 45·5, $P = 0{\cdot}0088$).

SEED GERMINATION

Total germination of non-ingested control seeds (64·0% ± 5·7 SD) was significantly higher than that of seeds that passed through the digestive tract of carp (41·9% ± 14·0 SD, overall effect of treatment ANOVA: $F_{1,13} = 7{\cdot}1$, $P < 0{\cdot}001$, post hoc tests of all activity levels to control seeds: $P < 0{\cdot}001$, Fig. 4a). Total germination (%) did not differ among the activity levels (*post hoc* Tukey contrasts among activity levels: all $P > 0{\cdot}81$).

In a second ANOVA, the interaction of activity treatment with retention time was included to test for an effect of seed retention time in the gut on the percentage of germinated seeds. Retention time did not have a main effect nor interacted with treatment in this model (main effect retention time: $F_{1,126} = 0{\cdot}025$, $P = 0{\cdot}87$, interaction effect: $F_{2,126} = 0{\cdot}098$, $P = 0{\cdot}91$).

However, the duration until germination (germination rate), was clearly different between controls and ingested seeds (Table 1, Fig. S4 and Table S2). Germination was significantly faster in control seeds (mean germination time 9·7 days) than in any of the seeds excreted by fish (mean germination time of 13·2 days, GLMM 4a in Tables 1 and S2). Germination rate of ingested seeds, however, did not differ among the activity levels, nor was affected by

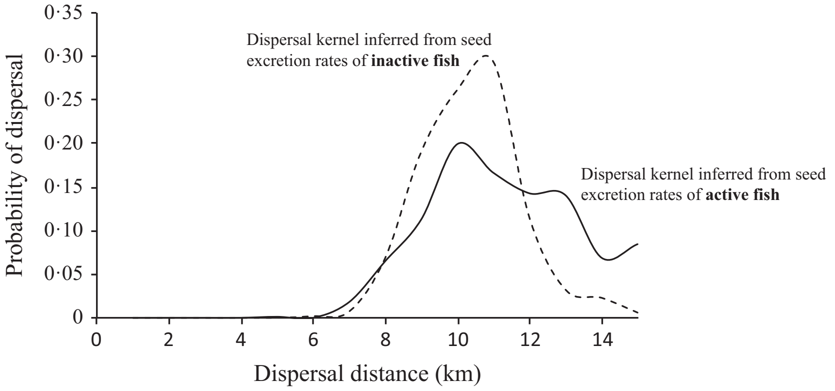

Fig. 3. The effect of physical activity of carp on the shape of the seed dispersal kernel. The dispersal kernels were modelled based on seed excretion rates over a period of 15 h (data for *Carex acuta* and *Carex riparia* were pooled) of physically active and inactive carp, respectively, in combination with non-stop, linear swimming at a constant optimum speed of one body length per second (1·0 body length s^{-1} = 0·277 m s^{-1}). To correct for potential biases towards the right limit of the observation intervals, we assigned excretion events to the mid-value of each time interval. Note the difference in the shape of the two dispersal kernels: the dispersal kernel based on seed excretion rates of active fish has a relatively fatter tail, indicating a larger potential for long-distance dispersal (Nathan *et al.* 2008). Our study suggests that modelling dispersal based on experiments with inactive fish (such as in our low-activity treatment) can lead to an underestimation of potential dispersal distances.

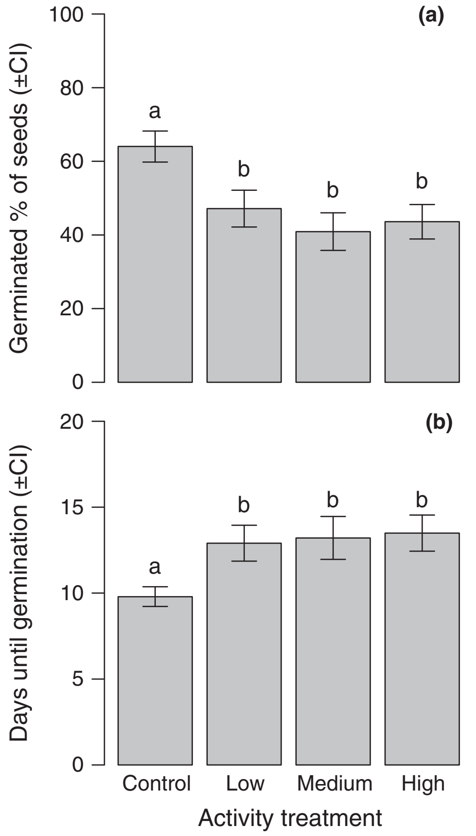

Fig. 4. (a) the mean percentage of seeds (*Carex acuta* only) that germinated and (b) the time in days until they germinated. Depicted for the non-ingested control seeds, and for the low-, medium- and high-activity treatments. Bars that do not share a common letter are significantly different. Control seeds germinated more and faster, but no differences were found between the levels of activity.

retention time of seeds in the fish (GLMM4b in Tables 1 and S2, Fig. S4).

Discussion

Physical activity during digestion affected the temporal pattern of seed excretion in fish. Active animals digested a similar proportion of the ingested seeds, but started excreting seeds later and kept excreting ingested seeds for at least 2 h longer. This is in accordance with the locomotion-priority mode hypothesis (compare Fig. 1 and Fig. 2) and suggests that the active fish redirected blood flow from their digestive system to their axial muscles during locomotion. Germinability of the seeds decreased due to gut passage through the fish compared to non-ingested controls, but this effect did not depend on the level of physical activity.

To illustrate the magnitude of our measured effect on seed dispersal, we constructed maximum potential seed dispersal kernels for active and inactive fish. Because retention time did not affect germination (beyond the effect of gut passage), these kernels illustrate the dispersal potential of viable seeds. As visualized in Fig. 3, the median moment of seed retrieval from active fish was on average 1 h (or about 9·3%) later compared to inactive fish. This is visible as the earlier decline of the probability of dispersal in active fish. Changes also occurred in the tail of the dispersal kernel. The upper quartile of the seed retrieval was delayed by about 1 h due to activity, corresponding to a 8·4% shift in timing of seed retrieval. At the end of the active period (t = 15 h), the fish subjected to the activity treatments were still defaecating seeds, while the excretion curve of the low-activity treatment fish had already plateaued. Hence, active fish excreted seeds later and had a fatter dispersal kernel, with longer potential dispersal distances of viable seeds (Nathan *et al.* 2008). We argue that previous studies using data from resting fish to model seed dispersal kernels probably structurally underestimated potential dispersal distances.

DISPERSAL DISTANCES BY ACTIVE VS. INACTIVE FISH

Figure 3 shows the dispersal kernels for both active and inactive fish obtained by combining information on seed excretion rates with literature information on the swimming speed of carp (Pollux *et al.* 2007; Pollux 2011). A study by Ohlberger, Staaks & Holker (2006) shows that the optimum swimming speed (U_{opt}), defined as that at which the energy required per unit of distance travelled is minimized, for carp that have a body mass between 500 and 600 g is close to 1 body length s^{-1}. The dispersal kernels depicted in Fig. 3 assume non-stop, linear swimming at a constant optimum speed of 1 body length s^{-1} (0·277 m s^{-1}) and provide information about the maximum potential distances over which carp can disperse seeds. Actual dispersal distances in the field are likely to be different, because carp may sometimes rest, may change speed or direction while swimming, or may swim along with or against currents.

Radiotelemetry studies report highly variable daily migration rates for carp, ranging from 0·147 to 21·6 km day^{-1} (Økland *et al.* 2001; Stuart & Jones 2006; Jones & Stuart 2007, 2009). Notably, the maximum potential distance inferred from our model based on only 15 h of monitoring active fish (~15 km per 15 h) could easily be achieved in natural situations given the potential daily migration rates of carp reported in a recent radiotelemetry study conducted in a large Australian lowland river (~21·6 km day^{-1}, Jones & Stuart 2009). This indicates that our model is realistic, at least for migratory individuals. Our model is also ecologically relevant: despite that the inferred maximum (long distance) dispersal events may be rare and will depend on many other ecological aspects (e.g. the motivation of individual fish to undertake displacement after or during foraging, Clausen *et al.* 2002), even rare dispersal events over long distances can importantly contribute to large-scale processes such as regional plant population or meta-community dynamics (Cain, Milligan & Strand 2000; Nathan *et al.* 2003, 2008; Nathan 2006).

The cumulative excretion of seeds shifted towards longer retention times for more active fish (Fig. 3), which suggests that carp prioritized their locomotor system (e.g. the heart and muscles) over blood flow to their digestive system (Brouns & Beckers 1993). This physiological trade-off, coined the swimming-priority mode (Palstra & Planas 2013) or locomotion-priority mode in our terminology, leads to longer seed retention times during active movement. This has important implications for both the previous and future constructions of dispersal kernels that estimate the impact of endozoochory on plant communities and distributions. For fish, but likely additionally for other taxa, the direct integration of data from known seed feeding experiments (reviewed in Pollux 2011) into dispersal kernel model estimates is problematic. Current knowledge underestimated the potential distances that can be achieved during fish-mediated dispersal and consequently the potential importance of fish as long-distance dispersers.

This study furthermore shows that an increase in the activity of a fish does not simply linearly increase its seed retention time. While carp at medium-activity levels had clearly longer retention times than carp at low-activity levels, a further increase of their metabolic rates to high-activity levels did not simply linearly increase their retention times. For *C. riparia* seeds, the excretion pattern remained the same for medium- and high-activity fish, suggesting the carp′s physiology responded in a somewhat binomial way to activity. This suggests that both two-fold and three-fold increases in metabolic rate lead to prioritization of blood flow from the digestive system to the locomotor system, and both treatments lead to the passing of a 'metabolic threshold' needed for redirection of blood flow. However, the retention times of *C. acuta* did not clearly differ between low- to high-activity levels, despite a three-fold increase in metabolic rate (whereas it was clearly different between low- and medium-activity levels). It therefore remains uncertain how we can expect the level of activity to precisely affect retention times. Still, our overall results clearly imply that already mild exercise in animals can induce significant changes in their dispersal estimates and, hence, the potentially realized seed shadows.

SEED RETENTION TIME AND METABOLIC RATE: ECTOTHERMS VS. ENDOTHERMS

The finding that physical activity negatively affects digestive processes in the common carp is in agreement with previous studies on ectotherms. Redirections of blood flow from the digestive to the locomotor system due to locomotion have been observed in, for example, Atlantic cod *Gadus morhua* (Axelsson & Fritsche 1991; Blaikie & Kerr 1996), Sablefish *Anoplopoma fimbria* (Furnell 1987), Graceful crabs *Cancer gracilis* (McGaw 2007) and Burmese pythons *Python molurus* (Secor & White 2010).

These findings in ectotherms contrast with how we currently think locomotion alters gastric rates in most endotherms. An endotherm's metabolism will also increase with its activity level, shunting blood away from the gastric system. However, simultaneously, peristaltic movements in the digestive system that accelerate food processing likely also increase with physical activity. These opposing directions in which exercise may influence gastric rates have best been studied in humans, and more recently in mallards (*Anas platyrhynchos*). Mild physical activity by humans (e.g. walking a 5 km h^{-1} for 15 min) stimulates gastrointestinal transit, while heavy exercise limits this (Keeling & Martin 1987; Marzio *et al.* 1991; Oettlé 1991; Dainese *et al.* 2004). Mild physical activity in mallards was found to shorten retention times and increase seed survival (Van Leeuwen, Tollenaar & Klaassen 2012; Kleyheeg *et al.* 2015), in contrast to our findings in common carp.

We propose that the effect of activity on gastric rates of ectotherms and endotherms is contrasting because of fundamental differences in metabolic costs of their digestive systems. Ectotherms have a lower metabolic rate and lower maintenance metabolic costs than endotherms. Hence, the amount of energy that is needed to break down food before it can enter the bloodstream (the specific dynamic action or heat increment of feeding) is higher relative to their BMR in ectotherms (Hicks & Bennett 2004; Klaassen & Nolet 2008). As their digestive system needs relatively more energy compared to their BMR, in ectotherms, gastric evacuation slows down even at mild exercise. In endotherms, at least as long as the activity level remains mild, peristaltic stimulation is probably dominant over such a reduction in blood flow, which leads to faster excretion (Van Leeuwen, Tollenaar & Klaassen 2012; Kleyheeg *et al.* 2015).

Overall, our current knowledge predicts contrasting effects of locomotion on seed retention times between ectotherms and endotherms. However, species-specific responses can also be expected within these groups. Species traits such as body mass, diet, optimal locomotor speeds or BMR may result in highly taxa-specific modulation of digestive processes by physical activity. Analogously, even within species BMRs will vary with changes of the environmental temperature, daily activity, seasonal variation, body composition and individual feeding behaviour. We therefore expect activity to modulate dispersal kernels in a broad range of animal species, and call for more future work on this topic to improve our current estimates of seed shadows by zoochory.

SEED GERMINATION

We found a negative effect of gut passage through fish on both total germination (%) and germination rate (expressed as the number of days until germination) of *C. acuta* seeds. The clear differences in germination between non-ingested controls and fish-ingested seeds show that gut passage influences seed viability. Similar effects have been observed in other aquatic plants (Smits, Van Ruremonde & Van der Velde 1989; Pollux *et al.* 2006;

Anderson, Rojas & Flecker 2009). Retention time in the digestive tract of carp did not affect germination (%) or germination rate.

This contrasts with studies on waterfowl where retention time generally negatively affects germination percentage (Charalambidou, Santamaría & Langevoord 2003; Charalambidou *et al*. 2005; Pollux, Santamaría & Ouborg 2005). Retention time is most likely less important in carp compared with waterfowl for two reasons: (i) carp have far shorter retention times than most birds (maximal 24 h in carp vs. 72 h in ducks; e.g. Pollux *et al*. 2006, 2007; García-Álvarez *et al*. 2015), which may limit the damage that can be inflicted to seeds, and (ii) carp have relatively shorter and simpler digestive tracts (e.g. lacking a stomach) than waterfowl which have specializations for processing food (a gizzard that is important for mechanical grinding and survival of dispersable seeds, see Charalambidou *et al*. 2005). Finally, we found no effect of swimming activity of carp (low, medium or high) on the germination (%) or germination rate of retrieved *C. acuta* seeds. The observed lack of differences between carp that were swimming at different speeds shows that the longer retention times observed in physically active fish are not associated with a reduction in seed viability. Therefore, while the seeds are dispersed farther, they have an equal probability to successfully germinate.

ECOLOGICAL RELEVANCE

To our knowledge, we showed for the first time that also actively swimming fish excrete viable seeds after long retention in their digestive systems. Our results indicate that fish may be even more important long-distance vectors for plants in river and pond systems than currently acknowledged. Thus, the role of fish as dispersal vectors that can counteract the drift paradox in river systems may also be stronger than previously estimated.

This study further improves our understanding of endozoochory as a dispersal mechanism, which is essential considering the increased need for plants to colonize new habitat in response to habitat loss and climate change. A crucial next step is to integrate experimentally obtained dispersal kernels with data on animal behaviour in their natural habitat. Increasingly realistic seed dispersal kernels should be constructed by combining our accumulating knowledge on animal foraging behaviour, animal movement data from telemetry studies and seed retention times obtained from active animals in experiments. Together, this will further enhance our understanding of how animals influence plant communities as ecosystem engineers.

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Data accessibility

Data deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.72ft2, (Van Leeuwen *et al*. 2015).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. Morphological characteristics of the two seed species, dimensions in mm ±SD and weight in mg ±SD, with significant differences indicated in boldface, $n = 20$.

Table S2. Model selection based on AICc values.

Fig. S1. Top view of flume tank, with indicated: (1) sieves to obtain the excreted seeds, (2) and (3) cages containing front and rear fish in the flume tank, respectively (4) outboard engines (5) biological filter continuously purifying the water.

Fig. S2. Box-and-whisker plots presenting the difference in retrieved percentage of seeds per retention time per treatment, in separate panels for *Carex acuta* (Panel 1) and *Carex riparia* (Panel 2).

Fig. S3. Retention times of fish subjected to low, medium or high activity treatments after being fed seeds from *Carex acuta* or *Carex riparia*.

Fig. S4. Cumulative germination of *Carex acuta* seeds subjected to the four different treatments.