SPECIAL REVIEW

The experimental study of seed dispersal by fish (ichthyochory)

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SUMMARY

1. The last few years have seen an increased interest in the experimental study of seed dispersal by fish (ichthyochory). This paper reviews such experiments, aiming to determine what functional aspects of ichthyochory have been investigated, what experimental designs have been used and what the potential pitfalls are.
2. The process of seed dispersal by fish can be divided into six discrete stages, each with its own probability of occurrence and each individually quantifiable in seed feeding trials: (i) seed uptake, (ii) ingestion, (iii) retention time, (iv) survival, (v) germination probability and (vi) germination rate after gut passage.
3. Inter- and intraspecific variation in seed traits (e.g. size, coat hardness, coat morphology, colour, presence and chemical composition of fruit pulp) and characteristics of fish (e.g. gape width, jaw morphology, presence of teeth, length of the digestive tract and digestive capability) can significantly affect the probability of one or more of the six stages of ichthyochory, thereby affecting the probability and distance of seed dispersal by fish.
4. To date only seven studies, which together investigated a total of nine fish species and 25 plant species, have used feeding experiments to study one or more of these quantifiable stages in the ichthyochory process. There is a clear bias in the research questions towards assessing seed survival during passage through the gut and subsequent viability. Only a few studies focus on seed retention in the digestive tract and germination rate, and even fewer address seed ingestion.
5. There is also considerable variation in experimental design among studies: Some have used groups of fish, while others used fish that are individually housed; some have fed seeds to hungry fish, while others used sated fish; some studied germination of seeds dissected from the alimentary tract, rather than seeds recovered from the faeces.
6. I present a number of recommendations for a more standardised protocol for future experimental studies of zoochory in general, and ichthyochory in particular, and highlight areas of interest for future research.

Keywords: retention time, seed ingestion, seed survival, seed viability, zoochory

Introduction

Dispersal plays a fundamental role in the life history of plants, affecting their biology, ecology, genetics and evolution (e.g. Fenner, 2000; Silvertown & Antonovics, 2001; Hanski & Gaggiotti, 2004). However, most plants lead a sessile life style and cannot move from

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one location to another. Instead, they rely on a number of external vectors, such as wind (anemochory), water (hydrochory) and/or animals (zoochory) to ensure the dispersal of their diaspores (Ridley, 1930; Van der Pijl, 1972).

The importance of zoochory in the dispersal of aquatic plants was recognised a long time ago (Darwin, 1859; Ridley, 1930). The diets of many water-associated animals consist, either partly or entirely, of seeds, which may be transported in the gut and subsequently dropped in new locations (endo-zoochory; Cook, 1988; Barrat-Segretain, 1996; Stiles, 2000; Levey, Silva & Galetti, 2002). Alternatively, seeds may adhere to the fur, feathers or feet of animals and thus be transported (ecto-zoochory; Sorensen, 1986; Cook, 1990; Smith & Stiles, 1994; Stiles, 2000). Waterbirds and fish are among the most likely candidates to play a role in the zoochorous dispersal of aquatic plants (Cook, 1988; Barrat-Segretain, 1996). The last decade has seen a rapid increase in interest in the ecological and experimental study of seed dispersal by waterbirds (e.g. Clausen *et al.*, 2002; Charalambidou & Santamaría, 2002; Figuerola & Green, 2002; Green, Figuerola & Sánchez, 2002), but seed dispersal by fish has not been subject to similar systematic investigation.

A number of findings suggest that seed dispersal by fish may play a significant role in the dispersal of aquatic and riparian plants. Field observations and stomach-content analyses of fish reveal that: (i) plant species are often consumed by many species; (ii) a significant proportion of fish may carry seeds in their digestive tract (depending on fish species, season and location); and (iii) the number of seeds found in fish guts may be substantial, ranging from a few to several hundreds or even thousands per stomach (e.g. Goulding, 1980; Kubitzki & Ziburski, 1994; Banack, Horn & Gawlicka, 2002; Mannheimer *et al.*, 2003; Piedade, Parolin & Junk, 2006; Correa *et al.*, 2007; Galetti *et al.*, 2008; Reys, Sabino & Galetti, 2009). In view of the prevalence of seeds in the digestive tracts of fish, along with the high density of fish in some systems (up to 10 000 per 100 m^{-2}; Henderson & Walker, 1990; Van Densen, Steinmetz & Hughes, 1990), it has been argued that fish may contribute significantly to the dispersal of aquatic and riparian plants (Pollux *et al.*, 2006).

Indeed, ichthyochory is often presumed to have important consequences for the ecology, distribution and persistence of vegetation along lakes and rivers. De Souza-Stevaux, Negrelle & Citadini-Zanette 1994)

argued that fish contribute to the upstream dispersal of many neotropical riparian plant species, because many fish are known frugivores and their upstream migration coincides with the annual flood during which seeds become more available. This idea gained further support through a study by Horn (1997), who: (i) using feeding experiments, showed that the Neotropical characid fish, *Brycon guatemalensis* Regan, consumed seeds of the riparian fig tree *Ficus glabrata* Kunth. and that the seeds retained the ability to germinate after gut passage; and (ii) using radio telemetry, confirmed that *B. guatemalensis* migrated upstream. Fish-mediated upstream dispersal of seeds may help maintain the longitudinal distribution of riparian plant species (e.g. *F. glabrata* along river systems of the Costa Rican rain forest; Horn, 1997), can help to maintain genetic and genotypic diversity within riparian plant populations (e.g. *Sparganium emersum* Rehmann along rivers in North-West Europe; Pollux *et al.*, 2007a, 2009a) and may contribute to the long-term persistence of aquatic and riparian plants in the headwaters (Pollux, Santamaría & Ouborg, 2005). Gottsberger (1978) takes these arguments one step further by arguing that the homogeneity of the plant community along Amazonian rivers and their tributaries is caused by the high frequency of fish-mediated seed dispersal.

Much of the information presently available supporting the idea that ichthyochory is significant for plant dispersal is based on field studies and dissections of fish collected from the field. Such studies have yielded valuable information on which fish act as dispersal agents and which plants are dispersed and have provided estimates of the frequency with which this occurs in nature. Moreover, these studies have given rise to other key questions pertaining to ecological and evolutionary interactions between plants and fish during fish-mediated dispersal. These include the following: Why are certain seeds more likely to be dispersed than others? How long do seeds remain in the digestive tract of fish? How does this affect their potential dispersal distance and/or viability after gut passage? What traits determine the probability that seeds survive gut passage? These questions can best be studied using an experimental approach, in which seeds are fed to fish under controlled (common-garden) experimental conditions. Here, I review studies that have used seed feeding trials to investigate the mechanisms underlying the

process of internal seed dispersal by fish, aiming to address the following questions: (i) What are the appropriate stages to measure in seed feeding experiments; (ii) How do seed traits and fish characteristics influence these stages; (iii) How do these stages determine the probability and distance of seed dispersal; and (iv) What are potential pitfalls that may arise during feeding trials? I also present a number of recommendations for a more standardised experimental protocol and highlight areas of interest for future research.

The different stages of internal seed dispersal by fish

To facilitate the experimental study of internal seed dispersal by fish, this process may be divided into a series of discrete, quantifiable, consecutive stages, each of which can be studied individually or in conjunction with the other stages. They are as follows (Fig. 1): (a) seed uptake ($P_{(u)}$), defined as the probability that seeds are taken into the mouth; (b) seed ingestion ($P_{(i)}$), the probability that seeds are swallowed; (c) seed retention time (RT), the time required for seeds to pass through the digestive system; (d) seed survival ($P_{(s)}$), the probability that seeds survive passage through the intestinal tract of an animal; (e) germination probability ($P_{(g)}$), the probability that seeds germinate after gut passage; and (f) germination rate after gut passage (GR), the time required to germinate (which may be inhibited, enhanced or unaffected compared to control seeds).

A seed may be subject to different selection pressures, depending on the preferences of the fish for particular fruit traits (e.g. seed size, seed coat hardness and morphology, colour and chemical composition of fruit pulp) prior to seed uptake (Fig. 1; *S1*) and seed ingestion (Fig. 1; *S2*), as well as on certain characteristics of the fish (e.g. gape width, jaw morphology, presence of teeth, length of the digestive tract, digestive capability) that affect its probability of surviving gut passage (Fig. 1; *S3*). After egestion, a seed may experience a different selection pressure compared to non-ingested (control) seeds, because gut passage may alter its susceptibility to pathogens and/or predators (e.g. antagonistic fungi, microbes and insects) because of damage of the protective seed coat (Fig. 1; *S4*). These different selection pressures on the seed will ultimately affect its probability of dispersal, dispersal distance, probability of establishment and subsequent seedling growth and survival as well as its ultimate lifetime reproductive success.

Seed uptake and seed ingestion

Seed consumption is the first stage in the dispersal process. When talking about seed consumption, it is helpful to distinguish between two sequential phases: (i) seed uptake, which I define as the transfer of seeds from the external environment into the mouth; and (ii) seed ingestion, defined as the subsequent transfer of seeds from the oral cavity to the digestive tract. Although the underlying mechanisms of selection during seed uptake and subsequent seed ingestion are likely to be very different, they have received little to no attention in experimental studies.

Seed uptake. Seed uptake can occur 'intentionally' or 'unintentionally' (Stiles, 2000). Some fish species may search for and pick up seeds actively when the
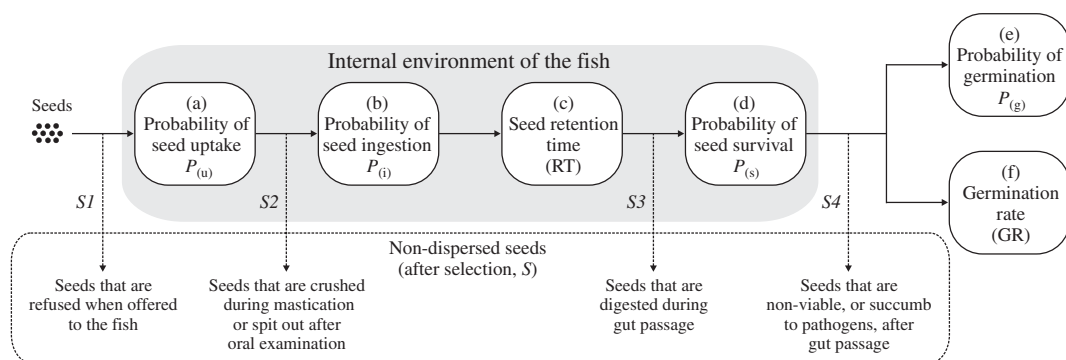

Fig. 1 Overview of the six discrete phases of the ichthyochoric dispersal process (a–f) and the opportunities for seed selection (S1–S4) (see text for details).

opportunity arises (i.e. facultative, opportunistic frugivores and granivores). Other species inadvertently take up seeds while searching for other types of food, for instance while foraging on vegetative plant parts (herbivores and omnivores), or while sifting through detritus for invertebrate prey (zoobenthivores and omnivores).

The way a particular fish species takes up seeds in the field will have important consequences for the design of feeding trials. Seeds can be fed directly to fish that intentionally eat seeds in the field (Adams *et al.*, 2007). However, fish that take up seeds unintentionally may not take seeds fed to them in a feeding trial. This problem has been reported in several studies and 'solved' by starving fish for several days before the experiment (Agami & Waisel, 1988; Smits, Van Ruremonde & Van der Velde, 1989). This may have two undesirable consequences: First, the physiology of hungry fish may be very different from that of sated fish, potentially affecting RT, survival and germination of seeds. Second, the refusal of fish to eat any of the seeds may lead to the erroneous interpretation that these fish do not function as important seed dispersers. For example, Smits *et al.* (1989) questioned the role of carp (*Cyprinus carpio* L.) in seed dispersal, based on such feeding trials, whereas field studies in Lake Banyoles (Spain) and the Carmargue (France) have shown that 42–93% of carp carry seeds in their stomachs, ranging from only a few to more than a 1000 per fish (Crivelli, 1981; Bergers, 1991; García-Berthou, 2001), highlighting the potential role of carp in the dispersal of seeds. Instead of starving fish prior to the feeding trial, it would be better to 'hide' the seeds in food before offering it, because this approach would better mimic the natural conditions of unintentional seed uptake (e.g. Horn, 1997; Pollux *et al.*, 2006, 2007a,b).

The opportunity for seed selection prior to uptake (Fig. 1; *S1*) also depends on whether fish take up seeds intentionally or unintentionally. During intentional seed uptake, there is ample opportunity for active selection by the fish, based on chemical (smell) or visual cues (size, shape or colour) (Araujo-Lima & Goulding, 1997). Some fish are even known to be attracted to the sound of seeds falling into the water (Gottsberger, 1978). However, by definition, there can be no selection by fish that take up seeds unintentionally. The probability of accidental seed uptake depends on the likelihood of fish encountering seeds while feeding, which in turn depends partly on seed abundance (frequency-dependence uptake). Finally, fish size constrains the probability of seed uptake (through gape limitation), regardless of whether the seeds are taken up intentionally or unintentionally (Stiles, 2000).

Interestingly, the preponderance of intentional and unintentional uptake is likely to differ between temperate and tropical regions. While many tropical fish species are opportunistic frugivores that intentionally ingest seeds, seed ingestion by temperate fish appears to be predominantly unintentional. This distinction between tropical and temperate regions is arguably related to general differences in fruit types (Figuerola, Green & Santamaría, 2002). In tropical regions, the production of fleshy fruits is prevalent among aquatic and riparian plants and trees, and these are often actively sought out and consumed by fish (Gottsberger, 1978; Goulding, 1980; Kubitzki & Ziburski, 1994). Most temperate aquatic plants, on the other hand, produce hard, non-fleshy fruits with a very small proportion of edible pulp material (e.g. *Zannichellia*, *Ruppia*, *Potamogeton*, *Sparganium*, *Sagittaria*, *Scirpus*), leading primarily to unintentional uptake (Pollux *et al.*, 2006).

Seed ingestion. Before actual ingestion, seeds are subject to stringent selection in the oral cavity. Fish have highly complex food selection mechanisms, involving morphological and behavioural adaptations as well as mechanical and chemical senses, for the detection and investigation of potential food items (Sibbing, Osse & Terlouw, 1986; Sibbing, 1988; Callan & Sanderson, 2003). The oral cavity can thus be viewed as a second stage in the seed selection process. Here, edible items are separated from the remaining material. The edible items are then ingested, while inedible particles are expelled by 'spitting' (a reversed suction pump action of the orobuccal and opercular cavities; Sibbing *et al.*, 1986; Callan & Sanderson, 2003).

Seed selection in the oral cavity may be based on a number of morphological characteristics of the seeds and fruits (Fig. 1; *S2*). The presence of a fleshy, juicy and nutritious fruit pulp may encourage fish to swallow the seed (Adams *et al.*, 2007). On the other hand, secondary metabolites in the fruit pulp (which may negatively affect taste; Stiles, 2000) and external structures on seeds or fruits (e.g. spines, barbs, hooks,

prickles, bristles, etc.) may discourage their ingestion. Seed hardness and size may also affect the probability of ingestion. Smaller and/or softer seeds are more likely to be ingested than larger and harder seeds (Table 1), presumably because the latter may be identified inedible and expelled (Pollux *et al.*, 2006, 2007a,b). It has further been argued that dispersal is more likely by larger adult fish because smaller, younger individuals may not be able to swallow the seeds. For example, small (<20 cm) channel catfish (*Ictalurus punctatus* Rafinesque) feed on *Forestiera acuminata* Michx. Poir. fruits by taking them into their mouths, scraping off the pulp and then spitting out the seeds, while only the larger individuals (40–60 cm) are able to ingest the whole fruits (Adams *et al.*, 2007; Markwith *et al.*, 2009). Similarly, smaller juveniles of the fish species *Colossoma macroponum* Cuvier are more likely to crush the seeds with their multicuspid, molariform teeth, while big adults are more likely to swallow the seeds whole (Anderson, Rojas & Flecker, 2009; J. T. Anderson, pers. comm.). These studies show that in some fish, there may be gape-limited, size-selective seed ingestion. In these species, larger adults are superior dispersal agents compared to juveniles. Although these studies provide preliminary evidence that under experimental conditions, certain seed traits (such as size and morphology) can influence the probability of ingestion, further studies are needed to assess the generality of this phenomenon.

Seed retention time

Gastric evacuation rate and retention time. Retention time (RT) depends on the gastric evacuation rate (GER) of fish. The rate at which the stomach is emptied can be affected by a large number of variables. Fish are poikilotherms, and when the water temperature falls, the rate of food intake and digestion both decline (leading to lower GER) (Flowerdew & Grove, 1979; Persson, 1979; Jobling, 1980). The GER is also influenced by the chemical and physical composition of the food (e.g. size and consistency of food particles, surface-to-volume ratio, energy content, meal size) (Flowerdew & Grove, 1979; Persson, 1979; Jobling, 1987) and may vary considerably, both among and within fish species, depending on size and species-specific variation in digestive physiology (Flowerdew & Grove, 1979; Jobling, 1980).

The question of how these factors influence seed retention in fish has rarely been the focus of experimental study. Ingested seeds should, in theory, remain longer in the intestinal tract when water temperature is low, thus increasing the potential range of dispersal. Studies with birds suggest that variation in seed size, composition and structure can also influence RT (Wahaj *et al.*, 1998; Tewksbury *et al.*, 2008; Fukui, 2003; Soons *et al.*, 2008), although such effects have not been observed in fish (Pollux *et al.*, 2006, 2007a,b). Clearly, further research is required to examine how various environmental factors (e.g. water temperature) and seed traits (e.g. seed size, seed structure) but also fish characteristics (e.g. fish size, gut length, inter-specific variation in digestive physiology) influence RT of seeds in the digestive tract of fish.

Potential dispersal distance. Retention time determines the potential distance over which seeds can be dispersed by the animal – the longer seeds remain in the digestive tract, the greater their potential dispersal distance. In fish, the potential dispersal distance can be modelled by combining information on RT with information on swimming ability (Fig. 2). During sustained swimming, fish typically adopt an optimum speed (U_{opt}), defined as that at which the energy required per unit of distance travelled is minimised (Beamish, 1978). Optimum swimming speed differs among species, depending on size and body shape, but for most fish (both marine and freshwater), U_{opt} lies somewhere between one to three body lengths per second (bl s^{-1}) (Jobling, 1995). Such 'dispersal curves' (Fig. 2) assume non-stop, linear swimming and provide information about the maximum distance that fish will be able to disperse the seeds. Actual dispersal distances in the field, however, are likely to be shorter because fish sometimes rest and, when swimming, may change speed or direction (Gerking, 1953; Stott, 1967; Ovidio *et al.*, 2002; Stuart & Jones, 2006).

Seed survival during gut passage

Seed survival refers to the egestion (defaecation) of apparently intact diaspores; i.e. seeds that appear to have an undamaged seed embryo (note that the seed coat may be damaged, scarified or removed) after gut passage (Fig. 1). The survival of seeds within the gut will depend both on the structure of the protective

Table 1 Empirical evidence from feeding trials for ichthyochorous dispersal of seeds [$N_{seeds\ offered}$, the number of seeds offered to the fish; ingestion (%), the percentage of offered seeds that were ingested by the fish; RT_{max}, the maximum seed retention time (in hours); $N_{seeds\ survived}$, the number of seeds that were retrieved from the faeces; survival (%), the percentage of ingested seeds that survived gut passage; FI (%), the percentage of fish-ingested seeds that germinated after gut passage; C (%), the percentage of (non-ingested) control seeds that germinated; FI (days), the germination rate (in days) of fish-ingested seeds after gut passage; C (days), the germination rate (in days) of (non-ingested) control seeds]

Plant species	Fish species	N_{fish}	Notes	Ingestion		Retention	Survival		Germination percentage		Germination rate		References
				N_{seeds} offered	Ingestion (%)	RT_{max} (h)	N_{seeds} survived	Survival (%)	FI (%)	C (%)	FI (days)	C (days)	
Alisma plantago-aquatica L.	Rr	–	–	–	–	–	80	–	25	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)
Annona muricata L.	Cm	5	A	7	–	–	–	95.2 ± 4.8	–	–	–	–	Anderson et al. (2009)
Annona muricata L.	Cm	5	J	4	–	–	–	95.0 ± 5.0	–	–	–	–	Anderson et al. (2009)
Bactris bidentula Spruce	Cm	5	A	7	–	–	–	33.3 ± 33.3	–	–	–	–	Anderson et al. (2009)
Bactris bidentula Spruce	Cm	5	J	4	–	–	–	65.0 ± 12.7	–	–	–	–	Anderson et al. (2009)
Cayaponia cruegeri Naud.	Cm	5	A	15	–	–	–	100	–	–	–	–	Anderson et al. (2009)
Cayaponia tubulosa Cogn.	Cm	5	A	5	–	–	–	73.3 ± 6.7	–	–	–	–	Anderson et al. (2009)
Cayaponia tubulosa Cogn.	Cm	4	J	3	–	–	–	83.3 ± 9.6	–	–	–	–	Anderson et al. (2009)
Cecropia latiloba Miq.	Cm	5	A	200	–	–	–	91.2 ± 5.0	–	–	–	–	Anderson et al. (2009)
Cecropia latiloba Miq.	Cm	4	A	60	–	–	–	92.6 ± 3.7	–	–	–	–	Anderson et al. (2009)
Cecropia latiloba Miq.	Cm	5	J	60	–	–	–	83.8 ± 4.1	–	–	–	–	Anderson et al. (2009)
Crateva tapia L.	Cm	5	A	7	–	–	–	50.8 ± 3.1	–	–	–	–	Anderson et al. (2009)
Crateva tapia L.	Cm	5	J	4	–	–	–	42.5 ± 13.2	–	–	–	–	Anderson et al. (2009)
Duroia duckei Huber	Cm	5	A	100	–	–	–	100	–	–	–	–	Anderson et al. (2009)
Duroia duckei Huber	Cm	4	A	60–70	–	–	–	94.2 ± 2.9	–	–	–	–	Anderson et al. (2009)
Duroia duckei Huber	Cm	5	J	60–70	–	–	–	89.5 ± 4.4	–	–	–	–	Anderson et al. (2009)
Gunnera chilensis Lam.	Ca	–	–	–	–	–	–	–	0	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)
Ficus glabrata Kunth.	Bg	6	I, H	100	100	48*	–	87.9*	70.4	–	10.09	8.58	Horn (1997)
Forestiera acuminata (Michx.) Poir.	Ip	–	E	–	–	–	–	–	56†	18†	–	–	Chick et al. (2003)
Menyanthes trifoliata L.	Ca	–	–	–	–	3	–	–	~100	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)
Menyanthes trifoliate L.	Pf	–	–	–	–	1	–	–	~100	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)
Menyanthes trifoliate L.	Rr	–	–	–	–	1–1.5	–	–	~100	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)

Table 1 (Continued)

Plant species	Fish species	N_{fish}	Notes	Ingestion		Retention	Survival		Germination percentage		Germination rate		References
				N_{seeds} offered	Ingestion (%)	RT_{max} (h)	N_{seeds} survived	Survival (%)	FI (%)	C (%)	FI (days)	C (days)	
Morus rubra L.	*Ip*	–	E	–	–	–	–	–	77[†]	62[†]	–	–	Chick *et al.* (2003)
Najas marina + *Ruppia maritima* (mixed)	*Cc*	10	G, S(4), H	100	100	30[†]	–	5[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina + *Ruppia maritima* (mixed)	*Ci*	10	G, S(4), H	100	100	82[†]	–	35[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina + *Ruppia maritima* (mixed)	*Or*	10	G, S(4), H	100	100	52[†]	–	60	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (hard seeds)	*Ci*	10	G, F, H	50	100	49[†]	–	52[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (hard seeds)	*Ci*	10	G, S(4), H	50	100	40[†]	–	78[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (hard seeds)	*Or*	10	G, F, H	50	100	72[†]	–	82[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (hard seeds)	*Or*	10	G, S(4), H	50	100	65[†]	–	100[†]	16	0	–	–	Agami & Waisel (1988)
Najas marina L. (light seeds)	*Ci*	10	G, F, H	50	100	23[†]	–	15[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (light seeds)	*Ci*	10	G, S(4), H	50	100	40[†]	–	15[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (light seeds)	*Or*	10	G, F, H	50	100	52[†]	–	25[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (light seeds)	*Or*	10	G, S(4), H	50	100	40[†]	–	17[†]	0	0	–	–	Agami & Waisel (1988)
Najas marina L. (soft seeds)	*Ci*	10	G, F, H	50	100	44[†]	–	5[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (soft seeds)	*Ci*	10	G, S(4), H	50	100	15[†]	–	5[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (soft seeds)	*Or*	10	G, F, H	50	100	52[†]	–	34[†]	–	–	–	–	Agami & Waisel (1988)
Najas marina L. (soft seeds)	*Or*	10	G, S(4), H	50	100	20[†]	–	12[†]	0	0	–	–	Agami & Waisel (1988)
Nuphar lutea L.	*Cc*	4	I, S(5)	25	100	–	–	0	–	–	–	–	Smits *et al.* (1989)
Nymphaea alba L.	*Cc*	4	I, S(5)	25	100	–	–	0	–	–	–	–	Smits *et al.* (1989)
Nymphaea caerulea Sav.	*Pf*	–	–	–	–	1.5	20	–	55	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)
Nymphoides peltata (Gmel.) O. Kuntze	*Cc*	4	I, S(5)	25	100	–	–	0	–	–	–	–	Smits *et al.* (1989)
Potamogeton natans L.	*Cc*	4	I, S(5)	25	100	–	–	16	–	–	–	–	Smits *et al.* (1989)
Potamogeton obtusifolius Mert. et Koch.	*Cc*	4	I, S(5)	25	100	–	–	2	–	–	–	–	Smits *et al.* (1989)
Potamogeton pectinatus L.	*Cc*	4	I, S(5)	25	100	–	–	7	–	–	–	–	Smits *et al.* (1989)
Potamogeton polygonifolius L.	*Rr*	–	–	–	–	2	–	–	0	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)
Ruppia maritima L.	*Cc*	10	G, F, H	200	100	5[†]	–	2[†]	–	–	–	–	Agami & Waisel (1988)
Ruppia maritima L.	*Cc*	10	G, S(4), H	200	100	26[†]	–	5[†]	–	–	–	–	Agami & Waisel (1988)
Ruppia maritima L.	*Ci*	10	G, F, H	200	100	42[†]	–	52[†]	–	–	–	–	Agami & Waisel (1988)
Ruppia maritima L.	*Ci*	10	G,S(4), H	200	100	70[†]	–	40[†]	–	–	–	–	Agami & Waisel (1988)

Table 1 (Continued)

Plant species	Fish species	N_{fish}	Notes	Ingestion N_seeds offered	Ingestion (%)	Retention RT_{max} (h)	Survival N_seeds survived	Survival (%)	Germination percentage FI (%)	Germination percentage C (%)	Germination rate FI (days)	Germination rate C (days)	References
Ruppia maritima L.	*Or*	10	G, F, H	200	100	75†	–	100†	–	–	–	–	Agami & Waisel (1988)
Ruppia maritima L.	*Or*	10	G, S(4), H	200	100	76†	–	70†	12.2	0.5	–	–	Agami & Waisel (1988)
Sagittaria sagittifolia L.	*Rr*	–	–	–	–	2	6	–	67	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)
Sagittaria sagittifolia L.	*Cc*	12	I, F, H, T	50	83.15‡	18	–	20.97	25.04	72.33	9.6	4.5	Pollux *et al.* (2006)
Sparganium emersum Rehm.	*Cc*	12	I, F, H, T	50	56.16‡	20	–	38.58	83.27	70.67	6.5	8.6	Pollux *et al.* (2006)
Sparganium emersum Rehm. (small seeds)	*Cc*	12	I, F, H	50	79.89‡	14	–	23.64	84.88	40.91	5.4	8.0	Pollux *et al.*, 2007a,b
Sparganium emersum Rehm. (medium)	*Cc*	12	I, F, H	50	50.44‡	12	–	42.24	72.02	69.21	7.3	8.7	Pollux *et al.*, 2007a,b
Sparganium emersum Rehm. (large seeds)	*Cc*	12	I, F, H	50	28.58‡	14	–	71.03	81.68	76.56	7.6	10.1	Pollux *et al.*, 2007a,b
Sparganium simplex Huds. (=*S. emersum*)	*Rr*	–	–	–	–	–	–	–	0	–	–	–	Hochreutiner (1899; reference taken from Ridley, 1930)

Bg, *Brycon guatemalensis* Regan (machaca); *Ca*, *Cyprinus auratus* L. (goldfish); *Cc*, *Cyprinus carpio* L. (carp); *Ci*, *Ctenopharyngodon idella* Valenciennes (grass carp); *Cm*, *Colossoma macroponum* Cuvier; *Ip*, *Ictalurus punctatus* Rafinesque (channel catfish); *Pf*, *Perca fluviatilis* L. (perch); *Rr*, *Rutilus rutilus* L. (roach); *Or*, *Oreochromis* sp. (tilapia).

A, adult fish were used in the feeding trials; J, juvenile fish were used in the feeding trials; I, seeds were used in the feeding trials; I, seeds were fed to individually housed fish; G, seeds were fed to a group of fish kept in a community tank; E, seeds used in the experiment were extracted from the lower digestive tract (intestine); S (days), fish were starved prior to the experiment, the duration of which indicated between brackets (in days); F, fish were well fed prior to the experiment (i.e. sated); H, seeds were 'hidden' in bread or food pellets; T, equal number of *Sparganium emersum* and *Sagittaria sagittifolia* seeds were simultaneously fed to each fish.

*Experiment was terminated at 48 h.

†Values estimated from graphs.

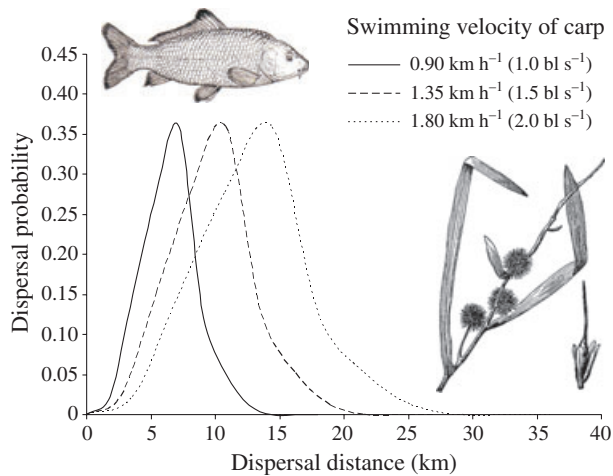‡Seed uptake was 100%, thus given values signify seed ingestion (%).

Fig. 2 Seed dispersal curves for *Sparganium emersum* when internally dispersed by the common carp (*Cyprinus carpio*). The three dispersal curves are based on seed retention times (at 24 °C) in the digestive tract of carp (tail length *ca.* 25 cm) and three different optimal swimming speeds of carp (U_{opt}, 1.0, 1.5 and 2.0 body lengths per second), assuming non-stop linear swimming over a period of 16 h. (redrawn after Pollux *et al.*, 2007a,b).

seed coat and the digestive capabilities of the animal (Fig. 1; S3) (Traveset, 1998; Traveset & Verdú, 2002).

Seed survival reported in experimental studies varies considerably among plant species. For example, percentage survival of seeds from 10 temperate plant species ranged from 0 to 67% when ingested by carp (*C. carpio*) (see Table 1 for details). Similarly, percentage survival from seven different neotropical plants fed to the fish *C. macropomum* varied from 33 to 100% (Table 1). The observed variation in percentage survival among plant species is probably related to the species-specific structure of the seed coat, which protects the embryo against mechanical forces and chemical digestion (Schupp, 1993). Indeed, the 'hardness' or 'toughness' of the seed coat, though generally undefined and unmeasured, is often invoked as the most important factor mediating interspecific differences in seed survival. Smits *et al.* (1989), for example, argued that the seed coats of three nymphaeids were too 'weak' to withstand digestion by carp, while two 'harder-seeded' *Potamogeton* species were able to pass intact (Table 1). Similarly, Pollux *et al.* (2006) showed that the 'hard' seeds of *S. emersum*, which have a tough corky exocarp and a hard scleridial endocarp, have a higher probability of survival than the 'softer', fleshy seeds of *Sagittaria sagittifolia* L. Even within plant species, differences in seed coats may produce differ-

ent survival probabilities. *Najas marina* L. produces three distinctly different types of seeds (hard, intermediate and soft). A comparative study showed that the soft and intermediate types survived less well than the hard type (Agami & Waisel, 1988). Another study showed that small seeds of *S. emersum* had a lower probability of survival than larger seeds, which presumably was related to differences in absolute seed coat thickness (Pollux *et al.*, 2007a,b). Finally, care should be taken when comparing survival probabilities obtained from different studies. While these differences in survival rate are probably at least partly related to differences in the structure of the seed coat, other confounding factors, such as differences in water temperature or fish size, may also have played a role.

Survival probability may also depend on the characteristics of the fish that consume the seeds. Differences among animals are likely to be related to variation in the morphology of the digestive system (e.g. length or complexity of the digestive tract) and/or physical and chemical environment in the digestive tract (Traveset, 1998; Schupp, 1993; Stiles, 2000; Charalambidou & Santamaría, 2002). To date, only one study has looked at differences in seed survival rate among fish species. Agami & Waisel (1988) investigated survival rates of *N. marina* and *Ruppia maritima* L. seeds ingested by three different fish species: the common carp, grass carp (*Ctenopharyngodon idella* Valenciennes) and tilapia (*Oreochromis* sp.). Their study revealed a strong effect of fish species on seed survival, with 5% in the common carp, 35% in grass carp and 60% in tilapia (Table 1). It is not clear whether these differences were because of species-specific variation in digestive capability among the fish or whether body size may have been a confounding variable (larger individuals will have longer intestines, thus retaining seeds in the gut for longer). Clearly, the variables that affect the probability of seed survival during fish-mediated dispersal are still poorly understood and require further study.

Seed germination after gut passage

Passage through the gut of vertebrate frugivores may either enhance or decrease germination of ingested seeds, as a consequence of: (i) the removal of the fleshy pulp, which may contain secondary metabolites that regulate seed germination (potentially enhancing or inhibiting germination); and (ii) the mechanical

and/or chemical treatment of the seed coat, which may subsequently facilitate seed imbibition (enhancing germination). Moreover, damage to, or removal of, the pulp and/or seed coat may alter the susceptibility of seeds to pathogens (fungal infections, microbes) or invertebrate seed predators, thereby further affecting the probability of germination after gut passage (Schupp, 1993; Cipollini & Levey, 1997; Traveset, 1998; Traveset & Verdú, 2002; Traveset, Rodríguez-Pérez & Pias, 2008).

To disentangle the confounding effects of pulp removal and mechanical and/or chemical treatment on seed germination, Samuels & Levey (2005) argued that the experimental design should encompass a comparison between: (i) intact fruits; (ii) manually extracted seeds (seeds whose fleshy fruit pulp has been manually removed or scarified); and (iii) seeds or fruits that have passed through the gut. A comparison between intact fruits and manually extracted seeds will reveal an effect of pulp removal; a comparison between intact fruits and egested seeds indicates an effect of gut passage (the combined potential effects of pulp removal and any additional treatment in the digestive tract); and a comparison between manually extracted and egested seeds can distinguish between the effects of pulp removal and any additional effect of gut treatment (mechanical scarification or chemical alteration of the seed coat or endocarp).

Germination percentage. Studies have shown that ingestion by fish may enhance the probability of germination of some plant species (Agami & Waisel, 1988; Smits *et al.*, 1989; Chick, Cosgriff & Gittinger, 2003; Pollux *et al.*, 2006; Anderson *et al.*, 2009), while having no effect (Horn, 1997; Adams *et al.*, 2007) or decreasing the probability of germination of others (Smits *et al.*, 1989; Pollux *et al.*, 2006; Anderson *et al.*, 2009). Enhancement of germination after passage through the gut is generally attributed to the removal of fruit pulp in species that have fleshy fruits (e.g. *Annona*, *Cecropia*, *Forestiera*, *Morus*; Anderson *et al.*, 2009) and to the scarification of the seed coat or endocarp in plant species that produce dry, hard, 'grain-like' fruits (e.g. *Najas*, *Potamogeton*, *Ruppia*, *Sparganium*; Pollux *et al.*, 2006), while a decline in germination is attributed to damaging of the seed embryo (typically in plant species that lack a hard protective seed coat, e.g. *Nuphar*, *Nymphaea*, *Nymphoides*, *Sagittaria*; Smits *et al.*, 1989; Pollux *et al.*, 2006).

Some studies have reported contrasting effects of ingestion by fish on germination. Chick *et al.* (2003), for example, showed that a significantly greater proportion of *F. acuminata* seeds germinated after ingestion by channel catfish compared with non-ingested control seeds, whereas Adams *et al.* (2007) found that neither manual pulp removal by the authors, seed handling by the channel catfish (e.g. taking up the seeds into their oral cavity, scraping off the pulp and spitting them out), nor gut passage by the fish influenced germination of *F. acuminata*. Anderson *et al.* (2009) reported contrasting effects on the germination of *Duroia duckei* Huber after ingestion by *C. macropomum*; they found that passage through the gut increased the probability of germination in one experiment, but decreased it in a second. The reasons for these contrasting results are not clear, but might be related to small sample sizes, the history (origin) of the seed populations and/or the method of seed storage prior to the experiment.

Germination rate. A few studies have looked in detail at the effects of fish ingestion on germination rate. Gut passage accelerated the germination rate of *S. emersum* and *Cecropia latiloba* Miq. compared to non-ingested control seeds, significantly delayed germination rate of *F. glabrata* and *S. sagittifolia* and had no effect on the germination rate of *Annona muricata* L. (Horn, 1997; Pollux *et al.*, 2006; Anderson *et al.*, 2009). Anderson *et al.* (2009) followed the experimental design advocated by Samuels & Levey (2005), which helped them to separate the effects of pulp removal and other effects of passage through the gut on the germination rate. They found that the germination rate of *C. latiloba* seeds ingested by fish was similar to that of control seeds without fruit pulp, but significantly faster than control seeds with pulp, suggesting that in this species, enhancement of germination after gut passage was mainly because of the removal of the pulp. By contrast, they found that the seeds of *Cayaponia cruegeri* Naud ingested by fish had similar germination rates to control seeds with pulp, but significantly faster germination than control seeds without fruit pulp, suggesting that in this species, pulp removal may delay germination.

Thus, ingestion by fish appears to influence the germination rate of at least some, if not most, aquatic and riparian species, but whether these effects actually affect fitness of the seedlings in the field is still

unknown. A meta-analysis showed that early emergence conveys selective benefits to the plant, such as faster seedling growth and a higher fecundity later in life, but also that it may ultimately have little effect on seedling survival (Verdú & Traveset, 2005). Verdú & Traveset (2005) cautioned that germination rates obtained under controlled experimental conditions may differ significantly from those measured in the field, because (i) seeds may experience harsher, less favourable conditions in the field, decelerating the germination of both ingested and non-ingested seeds, effectively reducing the difference between them; and (ii) the influence of local heterogeneity (on a 'micro-site scale', i.e. over millimetres or centimetres) in physical and environmental conditions and resource availability among seedlings in the field may override any potential (dis)advantages of fish ingestion on germination rate and seedling growth (Rodríguez-Pérez, Riera & Traveset, 2005; Verdú & Traveset, 2005). Moreover, in some cases, the change in germination rate under controlled conditions may be too small (e.g. a 2.1- day acceleration in the germination rate of *S. emersum* and a 5.1- day delay for *S. sagittifolia* under controlled conditions; Pollux *et al.*, 2006) to lead in the longer term to increased or decreased seedling performances (Figuerola *et al.*, 2005). Finally, early seedlings may experience a higher risk of mortality; for example, because they may be exposed to unpredictable and/or harsher environmental conditions early in the season or be subjected to a longer grazing period by herbivores (Figuerola & Green, 2004; Verdú & Traveset, 2005). Thus, care should be taken, when interpreting changes in germination rates because of gut passage found under controlled experimental conditions, in ascribing beneficial or detrimental effects to plant fitness in the field.

Potential relationships between ichthyochory and other modes of dispersal

It is certainly possible that ichthyochory is merely one of several dispersal phases in the life of a seed. Studies have shown that seed dispersal often follows a multi-phased process (polychory), each involving a different dispersal agent (Chambers & MacMahon, 1994; Vander Wall & Longland, 2004). This multi-phased dispersal process may be a common means of seed dispersal, both in temperate and tropical communities (Vander Wall & Longland, 2004). Fish may contribute

to polychoric dispersal by functioning as one of the vectors in the process. There is some experimental evidence for two such multi-phased processes: (i) hydrochory–ichthyochory and (ii) ichthyochory–ornithochory.

Hydrochory and ichthyochory

Hydrochory (water-mediated dispersal) is considered to be the most important dispersal mechanism for aquatic and riparian vegetation (Nilsson *et al.,* 1991; Boedeltje *et al.,* 2003; Riis & Sand-Jensen, 2006; Pollux *et al.,* 2009a,b). During water-mediated dispersal, seeds may be consumed and then dispersed by fish. Such secondary dispersal could have a positive influence on the spread of seeds for two reasons. First, if seeds sink in the deeper parts of lakes and rivers, they could be lost because they might not find the right conditions (e.g. temperature, light) for germination or for seedlings to survive. Fish could promote directed dispersal towards shallow water near the riverbank, lakeshore or in the inundated floodplain, that offers suitable conditions for germination and seedling survival (Adams *et al.,* 2007; Anderson *et al.,* 2009). Second, such targeted dispersal would be ineffective if seeds remained positively buoyant after gut passage. Feeding trials have shown that passage through the gut negatively affects the buoyancy of *S. emersum* and *F. glabrata* seeds, either because of the removal of the pulp or to scarification of the seed coat (Horn, 1997; Pollux *et al.,* 2007a,b, 2009a,b). Thus, fish not only transport seeds to suitable locations but also ensure that they sink and, hence, increase the probability they remain where deposited (though see Markwith & Leigh, 2008). The seeds of many hydrophytes, as well as most helophytes, need to be submerged to enable germination, and for these species, a negative influence on seed buoyancy after gut passage would confer an obvious additional advantage to ichthyochoric dispersal. However, the extent of this phenomenon still needs further investigation.

Ichthyochory and ornithochory

Another way in which ichthyochory can be part of a multi-step dispersal process is when fish-eating birds (e.g. cormorants, herons, kingfishers, pelicans, storks) consume seed-eating fish and then themselves

disperse seeds to other locations. Evidence that this actually occurs in nature is still largely anecdotal. Heslop-Harrison (1955; cited by Smits *et al.*, 1989) reported the presence of viable *Nuphar* seeds in the droppings of heron (*Ardea cinerea* L.), which had apparently eaten a fish that had previously consumed some *Nuphar* seeds. A recent study by Green *et al.* (2008) found 116 diaspores of four cosmopolitan plant genera (*Lemna*, *Myriophyllum*, *Nitella* and *Typha*) in a single 26.4 g faecal sample of the fish-eating Australian Pelican (*Pelecanus conspicillatus* Temminck) collected in the field. Apart from fish remains, this faecal sample contained four times more seeds than any sample from other waterbirds in the vicinity known to consume seeds (Grey teal, *Anas gracilis* Buller; Eurasian coot, *Fulica atra* L.; Black swan, *Cygnus atratus* Latham), suggesting that piscivorous birds may play an important role in the dispersal of seeds ingested by fish (Green *et al.*, 2008). Darwin (1859) was among the first (if not the first) to test this idea experimentally by feeding fish, with seeds in their stomach, to piscivorous birds:

Fresh-water fish, I find, eat seeds of many land and water plants; fish are frequently devoured by birds, and thus the seeds might be transported from place to place. I forced many kinds of seeds into the stomachs of dead fish, and then gave their bodies to fishing-eagles, storks, and pelicans; these birds, after an interval of many hours, either rejected the seeds in pellets or passed them in their excrement; and several of these seeds retained the power of germination.

Although the preliminary evidence is largely anecdotal, it lends compelling support to the idea that piscivores (e.g. predatory fish, fish-eating birds, otters) can contribute to plant dispersal by consuming seed-carrying fish. Future work should address this idea in more detail, for example, by means of a large-scale examination of faecal pellets from known piscivorous birds in the field (Green *et al.*, 2002) and/or comparative feeding experiments in which seeds from different plant species are fed to different fish which are then fed to different fish-eating birds.

Conclusions, recommendations and future research

I have tried to shed some light on the seed traits and fish characteristics that influence successful seed dispersal by fish, as inferred from experimental studies that have used seed feeding trials. To date, seven studies, which together investigated a total of nine fish species and 25 plant species (Table 1), have used feeding experiments to address various aspects of ichthyochory. Among these studies, there was a clear bias in the research questions towards assessing seed survival during gut passage and subsequent seed viability. Only a few studies have focussed on seed RT in the gut and germination rate, and even fewer have centred on seed ingestion. Hitherto, no study has investigated seed uptake. There has also been considerable variation in study design among the studies: For example, some used groups of fish kept together, while others used individually housed fish; some fed seeds to hungry fish, while others used sated fish; and some studied germination of seeds that were dissected from the alimentary tract, rather than seeds recovered from the faeces.

Recommendations

Seed feeding trials provide a powerful tool for studying ichthyochory. To enable reliable comparisons of findings, future studies should follow a more standardised experimental protocol. A number of recommendations can be made. (i) Fish should be housed individually rather than in groups, because variation among fish is a potentially interesting and important variable. (ii) Information on fish size (length and mass) and water temperature during the experiment should be presented, because these factors can potentially influence the outcome of the experiment. (iii) Fish should not be starved prior to the experiment to induce seed uptake and seed ingestion, because this may alter their digestive physiology. (iv) Studies should differentiate between the probability of seed uptake and subsequent probability of seed ingestion, because these are distinctly different events with different underlying mechanisms of selection. To this end, seed consumption should be studied in two separate phases: First, seeds should be fed directly to fish to mimic intentional uptake, allowing the investigation of seed selection prior to uptake (Fig 1; *S1*); Second, seeds should be fed indirectly to fish (by hiding them in food pellets; Horn, 1997; Pollux *et al.*, 2006, 2007a,b) to mimic unintentional uptake, allowing the assessment of selection in the oral cavity (after uptake but before ingestion; Fig 1; *S2*). (v) To separate the effects of mechanical (pulp removal, scarification)

and chemical treatment during gut passage on germination; the germination trials should follow Samuels & Levey's (2005) experimental design, in which the probability of germination of seeds that passed through the gut of fish is compared to both intact fruits and manually extracted seeds (seeds whose fleshy fruit pulp has been removed or whose seed coat has been scarified). In addition, seeds that do not germinate during germination trials should be tested for viability by means of tetrazolium tests (DeVlaming & Proctor, 1968) to distinguish between non-viable (dead) and viable (dormant) seeds. (6) Preferably, experimental studies should address all the phases of the dispersal process, because each may ultimately affect the fitness of the seed (Fig. 1).

Directions for future research

Seed feeding experiments lend themselves particularly well to examine ichthyochory in a comparative framework. Of particular interest are comparisons among plant species and among populations within plant species that differ markedly in their seed traits (e.g. size, seed coat thickness, hardness and morphology, pulp characteristics). A particularly strong study design to test correlations between seed traits, on the one hand, and the different stages of the dispersal process (Fig. 1a–f), on the other, would include plant species (or plant populations) whose phylogenetic relationships are well resolved, allowing for the application of phylogenetic comparative methods (e.g. phylogenetically independent contrasts; Felsenstein, 1985). It would furthermore be interesting to compare fish that differ in specific traits, such as gape width, size, gut anatomy, ontogenetic stage (juveniles *versus* adults) and diet (frugivores *versus* granivores, herbivores, omnivores and zoobenthivores).

Secondly, aquatic plants are often dispersed by more than one dispersal agent (wind, water, fish, birds, rodents) in a multi-phased polychoric process. Therefore, future studies should focus on the possible interactions between ichthyochory and other modes of dispersal. One area of particular interest is the possibility of seed dispersal by fish-eating animals that consume seed-eating fish (e.g. predatory fish or fish-eating birds; Green *et al.*, 2008).

Finally, in addition to consuming seeds and fruits, fish may also consume vegetative propagules (e.g. tubers, turions, bulbils, stolons, rhizomes and vegeta-

tive plant fragments; David & Sutton, 1996). There is anecdotal evidence that suggests that vegetative propagules may survive gut passage by waterfowl (Joyce, Haller & Colle, 1980). If this is true, then vegetative propagules are also likely to survive passage through the digestive tract of fish, because the fish gut is less specialised and less 'aggressive' than that of waterfowl (Pollux *et al.*, 2007a,b). Future studies should therefore also focus on the potential role of fish in the dispersal of vegetative propagules.

Acknowledgments

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