SHORT RESEARCH NOTE

Field evidence of dispersal of branchiopods, ostracods and bryozoans by teal (*Anas crecca*) in the Camargue (southern France)

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Abstract Invertebrate propagules may survive internal and external transports by waterbirds, thus facilitating their dispersal between aquatic habitats. However, field data on such transport remain limited, especially for exozoochory. We quantified and compared the rates of internal and external invertebrate transports simultaneously in a wintering population of teal (*Anas crecca*) in the Camargue (southern France). We inspected lower gut (rectum) contents of birds that had been shot ($N = 366$) and washed birds that had been live-trapped ($N = 68$) during the winters 2006–2007 and 2007–2008. At least one propagule was recorded in 2.5% and 10.3% of internal and external samples, respectively. Cladoceran, ostracod, plumatellid bryozoan and anostracan propagules were all recorded in both internal and external samples. Hatching confirmed their viability, except for anostracan eggs. At least three cladoceran species and two ostracod species were recorded, none of which had previously been shown to be dispersed by birds. Amongst external samples, we recorded a significant seasonal trend in 1 year with most propagules recorded before December, keeping with a seasonal decline in the availability of propagules on the water surface. This study provides evidence that teal may be important vectors of invertebrate dispersal both within the Camargue and along migratory flyways.

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Freshwater invertebrates occur in isolated habitats surrounded by unsuitable terrestrial landscapes. Despite this lack of obvious connectivity among sites, many freshwater invertebrates have broad geographical areas (Bănărescu, 1990; WCMC, 1998). Some organisms achieve wide distributions through active means such as flight at the imago stage. Many, however, are incapable of dispersing themselves and rely on agents such as animal vectors, wind or water flow (Bilton et al., 2001).

In recent years, important advances have been made in the study of passive dispersal of aquatic invertebrates by waterbirds (see Green & Figuerola, 2005, for review). Nevertheless, our knowledge of this process remains in its infancy compared with, for example, the understanding of seed dispersal by birds in terrestrial systems (Dennis et al., 2007). At a broad taxonomic scale, it is becoming apparent which groups of metazoans are most frequently dispersed by aquatic birds (Frisch et al., 2007). Nevertheless, at a fine taxonomic scale, the list of species or genera known to be dispersed by birds in the field remains extremely short, mainly due to the small number of studies, but also due to the difficulties inherent in identifying invertebrate propagules. Largely due to the greater ease with which seeds can be identified, there is much more taxonomic information available as to which plants are dispersed by waterbirds (Green et al., 2002; Brochet et al., 2009a).

Passive transport can be achieved by attachment of resting stages or of individuals on the outside of waterbirds ('exozoochory', 'epizoochory' or external dispersal), or through their transport within the guts of birds ('endozoochory' or internal dispersal) (Bilton et al., 2001). Several recent studies have focused on internal transport (e.g. Green & Sánchez, 2006; Frisch et al., 2007; Sánchez et al., 2007; Green et al., 2008). However, much less is known about external transport (Figuerola & Green, 2002a, b).

In this study, we investigated the potential of Common teal (Anas crecca) wintering in the Camargue (southern France) as a vector of invertebrate dispersal. We quantified the presence of invertebrate propagules transported externally and internally and we tested their viability. We also considered whether or not the rate of transport changed during the course of the winter, due to seasonal variation in the abundance and distribution of propagules.

During the winters of 2006–2007 and 2007–2008, we collected the final part (rectum) of the digestive tract from teal hunted from September to January ($N = 366$) to study internal transport, as well as samples from live teals caught for ringing purposes ($N = 68$) to study external transport (Fig. 1). Teal shot were flying out of feeding sites towards roosting sites, so that viable propagules in their rectum would very likely have been dispersed from one wetland to another. External samples were taken from teal caught from October to March using baited funnel traps. This took place in a day-roost deserted by teals during the night, so that viable propagules collected on the outside of these birds were also likely to have been dispersed during subsequent commuting flights to nocturnal foraging sites. Samples were first taken from the feet by rinsing both of these into a plastic container and then from the feathers by brushing the wings, tail and body into a tray with a nylon brush and then rinsing this material into a second plastic container. Figuerola & Green (2002b) found experimentally that almost all Daphnia ephippia placed in teal plumage are removed by such a protocol. Both internal and external samples were used in a separate study of seed dispersal (Brochet et al., 2009b).

In the laboratory, samples were rinsed with bottled mineral water (Volvic water, Volvic, France; conductivity: 170–200 µS/cm) through a 63-µm sieve. Smaller propagules (e.g. rotifer eggs) were lost at this stage. The residue was examined under a binocular microscope (Zeiss Stemi 2000, maximum magnification: ×50). Invertebrate propagules were separated from the residue, counted and identified using available literature (e.g. Vandekerkhove et al., 2004, for ephippia). Propagules were then left to hatch in mineral water at room temperature and natural light schedule, and checked on days 1, 3, 7 and then weekly for up to 56 days.

Given the difficulties in spotting invertebrate propagules among debris when inspecting samples, during the second winter, after searching through samples to pick out propagules, we also carried out emergence tests for 96 internal and 45 external samples (52% and 78% of samples from that winter, respectively), by inundating the remaining residue of each sample in a 120 cm^3 vial together with 50 cm^3 of mineral water to permit hatching of any remaining propagules. This could not be done for all samples due to lack of laboratory space. Vials were covered,

Fig. 1 Map of the
Camargue showing the
seven collection sites for
rectum samples (*dark
circles*) and the collection
site for external samples
(*hatched square*)
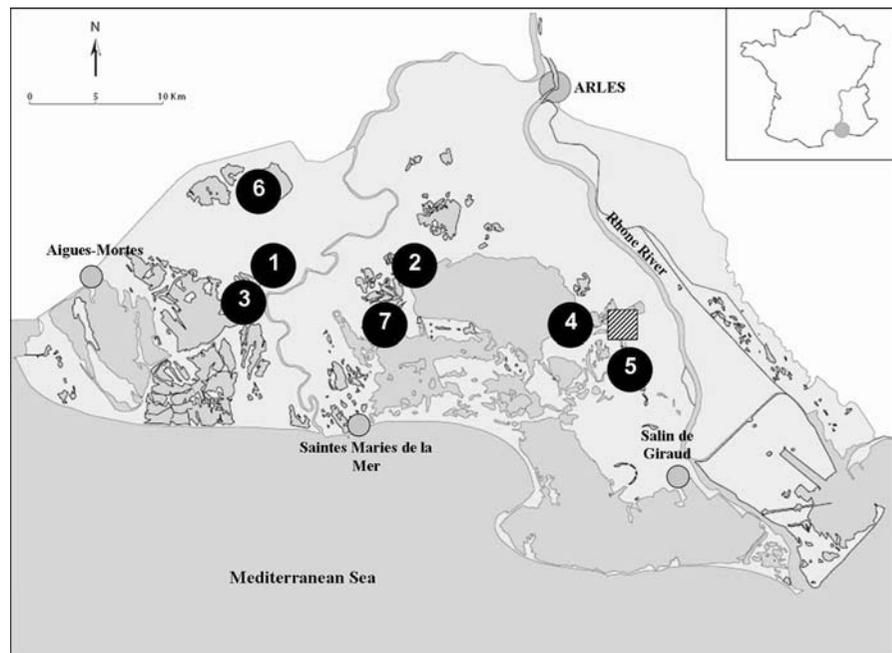
allowing air exchange but reducing evaporation and
the risk of aerial contamination by other propagules.
Twenty four controls (one per week for the period of
sample analysis) were established with mineral water
only. Vials were randomly placed on the laboratory
bench with a natural light schedule and temperatures
oscillating from a minimum of 12°C at night to a
maximum of 28°C during the day. The presence of
live invertebrates was checked on days 1, 3, 7 and
then weekly for up to 56 days. Live invertebrates
were separated from the residue, counted and
preserved in alcohol for later identification. Unfortu-
nately, some ostracods were preserved as early instars
that could not be identified to the species level.

The sampling period was divided in two halves:
before and after 25 November (approximately the
middle of the wintering season). We carried out
2×2 Fisher exact tests to test the differences in
numbers of samples with at least one invertebrate
present (as hatched or unhatched propagules) in the
early and late part of the winter season, for each
winter separately and for both rectum and external
samples. The presence–absence was used rather than
the numbers of animals recorded in a given sample,
because numbers of some taxa were likely to have
been increased by reproduction within the vials.
R software (version 2.8.1) was used for analysis
(R Development Core Team, 2008).

We found propagules of Anostraca, Cladocera and
Bryozoa in 1.4% of internal samples and 2.9% of
external samples (Table 1). We did not find more
than one propagule taxon in any single sample. Two
out of three *Plumatella* statoblasts hatched. One dead
but intact adult ostracod was also found in each of
two internal samples, but no resting eggs hatched
from these adults.

In emergence tests, 3 out of 96 rectum samples
(3.1%) and 5 out of 45 external samples (11.1%)
contained at least one hatched invertebrate (Table 2).
Cladocerans and ostracods hatched from both internal
and external samples. No more than one invertebrate
type was recorded from a given sample. No individ-
uals were detected earlier than 14 days after inunda-
tion (Table 2), indicating that all individuals hatched
from eggs. No invertebrates emerged from any
control sample. Overall, at least one Anostraca, three
Cladocera, two Ostracoda and one Bryozoa taxa were
recorded (Tables 1, 2).

In the second winter, external samples collected in
early winter were more likely to contain invertebrate
propagules (hatched and unhatched propagules) than
those collected in late winter (Fisher exact test:
$P = 0.009$). No such seasonal effect was apparent for
internal samples (Fisher exact test: $P = 1$). The
number of propagules detected in the first winter
(Table 1) was too low to permit statistical analysis.

Table 1 Invertebrate propagules found in teal rectum (internal) and feet and plumage (external) samples ($N = 366$ and 68, respectively) collected during winters 2006–2007 and 2007–2008

Sample type	Taxon		Location	No. of propagules	Occurrence (number of teal)	Year of collection	Month of collection
External	Anostraca	Unidentified anostraca	Plumage	1	1	2006–2007	February
	Bryozoa	*Plumatella* sp. (statoblast)[a]	Plumage	1	1	2007–2008	October
			Feet	1	1	2007–2008	November
Internal	Anostraca	Unidentified anostraca	Rectum	1	1	2006–2007	September
	Cladocera	*Ceriodaphnia* sp. (ephippium)	Rectum	1	1	2006–2007	November
			Rectum	1	1	2007–2008	November
	Bryozoa	*Plumatella* sp. (statoblast)[b]	Rectum	1	1	2006–2007	October
			Rectum	1	1	2006–2007	January
			Total	8	8		

[a] The statoblast found in October hatched after 21 days in mineral water

[b] The statoblast found in January was placed 3 months in a refrigerator to break dormancy and then hatched after 3 days in mineral water

Table 2 Invertebrates recorded during emergence tests, in teal rectum (internal) and feet and plumage (external) samples ($N = 96$ and 45, respectively), collected during winter 2007–2008

Sample type	Taxon		Location	No. of live individuals[a]	Occurrence (number of teal)	Month of collection	Day of observation
External	Cladocera	*Chydorus sphaericus*	Feet	13	1	October	21
	Ostracoda	*Potamocypris* cf. *producta*	Plumage	1	1	October	21
		Cyprididae	Plumage	1	1	October	42
		Unidentified ostracoda	Feet	1	1	October	14
			Feet	1	1	November	28
Internal	Cladocera	*Macrothrix* cf. *hirsuticornis*	Rectum	18	1	September	42
	Ostracoda	*Paralimnocythere* cf. *psammophila*	Rectum	1	1	January	21
		Cyprididae	Rectum	2	1	December	28
			Total	38	8		

Day of observation refers to the number of days between inundation of samples and the first observation of invertebrates

[a] These figures correspond to the number of individuals observed alive and may exceed the original number of propagules in the sample, since it is possible that they reproduced in the vials

As far as we know, this is the first study to quantify both external and internal transports of invertebrates simultaneously in the same bird population. In separate winters, Figuerola & Green (2002b), Figuerola et al. (2003) and Frisch et al. (2007) studied both internal and external transport by mallard *Anas platyrhynchos* and coot *Fulica atra* in Doñana, Spain, but did not test the viability of propagules transported externally.

We found anostracans, cladocerans, ostracods and bryozoans to be transported by both exo- and endozoochory. To our knowledge, there are no previous field demonstrations of external transport of ostracods and anostracans by birds, although Frisch et al. (2007) hatched ostracods from mud likely to be transported by birds. In addition, Vanschoenwinkel et al. (2008a) showed in the Camargue that ostracods are transported on the outside of wild boar *Sus scrofa*. Although we did not demonstrate it in the present study, live adult ostracods are sometimes excreted in duck faeces (Frisch et al., 2007). Our results, together with previous studies (Proctor, 1964; Figuerola et al., 2004; Frisch et al., 2007; Green et al., 2008), suggest that the

dispersal of ostracods, cladocerans and plumatellid bryozoans by waterbirds is a widespread phenomenon. In the case of ostracods and cladocerans, the genera recorded in the present study have not previously been cited in field studies of dispersal by birds, with the following exception: Proctor (1964) recorded *Macrothrix laticornis* in the lower intestine of gadwall *A. strepera*.

Our data probably greatly underestimated the importance of internal transport of invertebrates, due to the low probability of finding propagules in the rectum at the time of our snapshot sampling. Many more propagules present higher in the gut would very likely have survived gut passage if the teal had not been hunted. For instance, we found many intact cladoceran ephippia (mainly *Ceriodaphnia* sp.) in the upper gut (oesophagus and gizzard) of 12% (43 out of 366) of the same teal sampled here for rectum contents. These 43 teal contained on average 10 ± 3 [$x \pm$ s.e.] ephippia. We tested the viability of 110 of these ephippia (from 8 different birds collected during the second winter) and hatched *Oxyurella tenuicaudis* in one sample (Brochet et al., unpub. data). Exozoochoric dispersal rates were also likely to be underestimated in our study. It is possible that some propagules became detached from teal between entering the trap and being examined (birds were caught in the trap during the day and stayed there until dusk, then were transported in mesh cages from the trap within the marsh to the ringing site on the shore).

Our results on seed dispersal for the same birds (Brochet et al., 2009b) suggest that the probability of internal transport of seeds is higher than that of internal transport of metazoan invertebrates. In the Camargue, teal are highly granivorous during winter, and invertebrates have been estimated to represent only 5% of their diet (Tamisier & Dehorter, 1999). However, teal may be vectors for meiofauna such as copepods or nematodes which they ingest (Gaston, 1992) and whose small eggs may have been lost during sample processing. For the same teal samples, endozoochory of aquatic seeds was much more important than exozoochory (Brochet et al., 2009b), whereas the relative importance of these two dispersal modes for invertebrates is not clear from our results.

Our results suggest a seasonal trend in the probability of external transport of invertebrates by waterbirds, with higher chances of dispersal in early compared to late winter. For internal transport of invertebrate propagules, Figuerola et al. (2003) found that seasonal trends throughout winter are inconsistent between duck species and propagule types. Sánchez et al. (2007) found that internal dispersal rates of brine shrimp *Artemia* cysts were very high in both spring and autumn, but close to zero in midwinter. Since propagules can be ingested from propagule banks in sediments throughout the whole year, it is often hard to predict when internal transport is most likely to happen. In contrast, external transport is most likely when eggs can be concentrated at the water surface or along the shoreline and be readily picked up in feathers or on feet. For instance, at the start of inundation of a wetland/marsh, a lot of propagules can be floating (Brendonck & De Meester, 2003), and it has often been suggested that this is for promoting dispersal (see Vanschoenwinkel et al., 2008b). On the other hand, *Daphnia* often deposit their ephippia at the water surface (Ślusarczyk & Pietrzak, 2008). For cladocerans, ostracods and bryozoans in Europe, propagule release is concentrated in summer or autumn as conditions deteriorate (Ślusarczyk & Pietrzak, 2008; Hartikainen et al., 2009), making attachment to ducks more likely in our early than our late winter period. Moreover, in the Camargue as elsewhere, the densities of invertebrates often drop by midwinter (Dubowy, 1988; Guillemain et al., 2000).

Even if only a minority of birds were transporting invertebrate propagules, thousands of birds were dispersing invertebrate propagules on a daily basis within our study area. Teal are extremely abundant in the Camargue, representing 20–30% of wintering ducks with an annual peak count averaging 36,000 birds (Tamisier & Dehorter, 1999). They make regular movements of a few kilometres between different waterbodies, spending the day on roosting sites and moving to feeding grounds at night (Tamisier, 1978). However, long-distance movements also occur throughout the winter, and there is a high turnover rate within the teal wintering population (Pradel et al., 1997). There are approximately 2 million teal wintering in Europe (Wetlands International, 2006), many of which undergo long-distance migrations to the Camargue and other areas. This study suggests, therefore, that teal play a role in the passive transport of invertebrates within- and between wetlands. Cladocerans and ostracods can found

new populations from one single propagule via parthenogenesis and bryozoans via clonal reproduction.

Our results suggest exozoochory may play a relatively more important role during the southwards, autumn migration period than in midwinter and perhaps during northwards, spring migration. There are few data on how long invertebrates remain attached to birds, and the nature of dispersal distance for exozoochory is open to speculation (Green & Figuerola, 2005). Concerning internal transport, statoblasts and ephippia can be retained for up to 44 h inside duck guts, although most are defecated within 4 h of ingestion (Charalambidou et al., 2003a, b). As teal fly at ca. 58 km/h (Clausen et al., 2002), long distances can be covered during the retention time of ingested propagules (see Lebarbenchon et al., in press, for details of long-distance teal movements from our study site). Moreover, bryozoan colonies with identical multilocus genotypes were shared between sites along duck migration routes separated by 700 km of land and sea (Freeland et al., 2000).

In conclusion, we have shown that teal are vectors of various cladoceran and ostracod taxa not previously shown to be dispersed by birds. Our results also suggest the existence of a seasonal shift in the probability of exozoochory of invertebrate propagules by teal. The spatial patterns of teal movements combined with retention time of propagules suggest a high frequency of dispersal within the Camargue, as well as long-distance dispersal.

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