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Dispersal of aquatic invertebrates by lesser black-backed gulls and white storks within and between inland habitats

Víctor Martín-Vélez[1] · Marta I. Sánchez[1,3] · Ádam Lovas-Kiss[2] · Francisco Hortas[4] · Andy J. Green[1]

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Abstract

Waterbirds can transport aquatic invertebrates internally, contributing to metapopulation dynamics between aquatic habitats in a terrestrial matrix. However, research into this dispersal process to date has focused on individual field sites or laboratory studies. We investigated the invertebrates dispersed by endozoochory by the lesser black-backed gull *Larus fuscus* wintering in Andalusia, south-west Spain in 2016–2017, comparing seven sites interconnected by their movements, with different degrees of anthropogenization [three landfills, two saltpan complexes, a natural lake, and a large (370 km^2) ricefield area]. In the ricefields, we also compared invertebrates dispersed by gulls with those dispersed by the larger white stork *Ciconia ciconia*. A total of 642 intact invertebrates and their propagules (mainly plumatellid bryozoans, cladocerans, and other branchiopods) were recorded in excreta (faeces and pellets) from gulls and storks. A greater diversity and abundance of invertebrates were recorded in ricefields, notably 43 individuals of the alien snail *Physella acuta*. One snail was still alive in a gull pellet 3 weeks after being stored in a fridge. This represents the first record of snail dispersal within waterbird pellets. Viability was also confirmed for the cladoceran *Macrothrix rosea* recorded in ricefields, and the alien brine shrimp *Artemia franciscana* recorded mainly in saltpans. In ricefields, gulls and pellets had significantly fewer propagules and fewer taxa per gram of excreta than storks and faeces, respectively. Through their high mobility, gulls and storks can disperse invertebrates between different natural and artificial habitats, and even to landfills. They can promote metapopulation dynamics for native bryozoans and branchiopods, but also the spread of invasive snails and brine shrimp.

Keywords *Artemia* · Cladocera · Endozoochory · Gastropoda · Waterbirds

Introduction

Movement between isolated water bodies represents an important challenge for aquatic organisms. With the exception of adult insects, most aquatic invertebrates lack a capacity to move actively amongst wetlands, yet broad distributions and population genetic studies indicate that local and large-scale dispersal are widespread phenomena (Bilton et al. 2001; Tesson et al. 2015; Frisch et al. 2021). This also applies to alien invertebrates such as freshwater snails (e.g., *Physella acuta*) and brine shrimps (e.g., *Artemia franciscana*) (van Leeuwen et al. 2013; Horváth et al. 2018).

Since the pioneering studies of Darwin (1872), it has become widely accepted that waterbirds can transport aquatic organisms in their guts by "endozoochory", or externally by "epizoochory" (or "ectozoochory"), (Green and Figuerola 2005; Coughlan et al. 2017). They can also be important vectors for alien invertebrates (Green 2016). Compared with abiotic dispersal mechanisms (wind and water), waterbirds allow dispersal over longer distances, and often more directed towards suitable habitat (Parekh et al. 2014; van Leeuwen et al. 2012a; Viana et al. 2016).

Previous studies have demonstrated the capacity of a wide range of waterbirds to disperse freshwater

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✉ Víctor Martín-Vélez
victormartin_velez@hotmail.com

[1] Department of Wetland Ecology, Estación Biológica de Doñana, CSIC, Américo Vespucio 26, 41092 Seville, Spain

[2] Department of Tisza River Research, Wetland Ecology Research Group, Centre for Ecological Research, Bem tér 18/C, Debrecen 4026, Hungary

[3] Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, 41012 Seville, Spain

[4] Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional/Global del Mar (CEI·MAR), Universidad de Cádiz, Av. República Saharaui s/n. 11510, Puerto Real, Cádiz, Spain

invertebrates by endozoochory when ingesting resistant eggs of water fleas (freshwater cladocerans) or anostracans (fairy and brine shrimps), statoblasts of bryozoans (moss animals), or propagules of other organisms (Green et al. 2008; Sánchez et al. 2012; Okamura 2019; Silva et al. 2021; Briscoe et al. 2021). Sometimes, this happens when preying on primary dispersers such as fish or crayfish that are carrying the propagules (van Leeuwen et al. 2017; Lovas-Kiss et al. 2018), this being a form of "secondary dispersal". However, compared with recent advances in research into the role of waterbirds in the dispersal of plants (e.g., Lovas-Kiss et al. 2019; Martín-Vélez et al. 2021a; Sebastián-González et al. 2020), their role as dispersal vectors of invertebrates remains poorly investigated. For example, their importance as vectors for molluscs remains unclear, despite anecdotal observations and an increasing number of studies of mollusc genetics that support a key role for birds (Green and Figuerola 2005; Martin et al. 2020; Boulaassafer et al. 2020).

As yet, there are few studies comparing the rates of invertebrate endozoochory by different bird species with different morphologies in a given location (Sánchez et al. 2007; Green et al. 2008; Valls et al. 2017; Moreno et al. 2019), and even fewer comparing the rates of dispersal by a given species at different locations (Green et al. 2005). In Andalusia, southwest Spain, the lesser black-backed gull (LBBG) *Larus fuscus* is an opportunistic feeder which exploits and connects a wide range of habitats, including landfills, ricefields, coastal wetlands, and natural lakes (Martín-Vélez et al. 2020). The white stork *Ciconia ciconia* is another generalist feeder common in ricefields, landfills, and other agricultural landscapes (Tablado et al. 2010; Bécares et al. 2019). These are both migratory birds that are important members of the waterbird community in Andalusia, and whose numbers have increased markedly over the last 40 years (Rendón et al. 2008; Ramo et al. 2013). Outside the breeding period, ricefields are particularly important for both species, which feed mainly on alien crayfish *Procambarus clarkii*, and egest seeds in both their faeces and regurgitated pellets (Martín-Vélez et al. 2021a). Pellets contain undigested food items, and are normally produced at roosting sites at the end of the day. Shorter maximum gut retention times and dispersal distances are expected for propagules egested in pellets than for faeces (Martín-Vélez et al. 2021b). Like seeds, small invertebrate propagules stuck on the outside of crayfish are liable to be ingested and dispersed by these birds (Lovas-Kiss et al. 2018). Furthermore, waterbirds can directly ingest larger invertebrates such as snails.

In this study, we aimed to investigate the potential for invertebrate dispersal by *L. fuscus* and *C. ciconia* in Andalusia, through faecal and pellet sampling. On one hand, we compared the invertebrates dispersed by *L. fuscus* and the larger *C. ciconia* as they fed in the ricefields. On the other hand, in the case of *L. fuscus*, their movements between ricefields and other habitats in Andalusia have recently been studied in detail (Martín-Vélez et al. 2020), and we extended our study of invertebrate endozoochory to other connected habitats, including salt pans, natural lagoons, and landfills. Apart from the ricefields, landfills are the habitats that maintain most of the connectivity in the network of habitat patches (nodes) interconnected by direct flights (links) of *L. fuscus*, and are strongly connected to lakes, salt marshes, and other aquatic environments (Martín-Vélez et al. 2020). We compared the invertebrates egested by gulls at different locations with differing degrees of anthropogenic impact and known connections to other locations in the connectivity network (Martín-Vélez et al. 2020).

Our main objectives were (1) to determine the differences between bird species and sample types (pellets versus faeces) in the invertebrates dispersed within ricefields, the habitat supporting the largest numbers of birds; (2) to establish the differences in invertebrates dispersed by *L. fuscus* across different habitats, including landfills visited by gulls roosting in wetland habitats; and (3) determine the viability (and further identification) of aquatic macroinvertebrates by carrying out hatching experiments.

Methods

Study sites

This study was carried out across seven sites in Andalusia used for roosting and feeding by *L. fuscus*, including ricefields (where *C. ciconia* were also sampled), landfills, salt pans, and a natural lake (Table 1; Fig. 1), with a known and varying extent of connectivity through direct *L. fuscus* flights (Martín-Vélez et al. 2020, 2021b). These sites are described as follows (see Martín-Vélez et al. (2020, 2021c) for more details):

- The ricefields of the Guadalquivir delta of 37,000 ha, an important part of the Doñana wetland complex (Green et al. 2018). The seeds in these samples were described by Martín-Vélez et al. (2021a). Over 10,000 *L. fuscus* and over 1000 *C. ciconia* were present during our study.
- Cetina saltpan complex (in the Gulf of Cadiz; 1100 ha) was created in 2014 and is one of the most important in Spain for salt production. *Larus fuscus* use it mainly as a roosting site. In the Cadiz Bay, in which Cetina Salt pan is included, an average of 5882 individuals were counted.
- Fuente de Piedra lake (1350 ha) is the largest natural lake in Andalusia and is a roosting area for over 20,000 *L. fuscus* in winter. See Batanero et al. (2017) for more details of this habitat.

Table 1 Sampling dates and numbers of excreta samples (showing faeces and pellets separately) collected for gulls and storks in each site during the winters of 2016–2017 and 2017–2018

Sampling period	Punta Entinas saltpan	Cordoba landfill	Fuente de Piedra lake	Alcalá del Río landfill	Cetina salt pan	Rio Tinto landfill	Doñana Ricefields				Total
	Feb. 2017	Feb. 2017	Jan- Mar 2017	Feb. 2017	Feb. 2017	Jan. 2017	Nov. 2016		Sep.–Dec. 2017		
Species	*Larus fuscus*						*Ciconia ciconia*	*Larus fuscus*	*Ciconia ciconia*	*Larus fuscus*	
n Faeces	40	33	51	19	30	16	23	53	85	109	459
n Pellets	0	0	23	0	0	0	51	26	26	54	180
n Samples	40	33	74	19	30	16	74	79	111	163	639

- Punta Entinas Natural Reserve (785 ha) is a complex of coastal wetlands composed of dunes, hygrophilous vegetation, and saltpans that provide refuge to a variety of birds (Luque 2003). Over 1000 *L. fuscus* were counted within the saltpan.
- Three landfill sites (Río Tinto landfill in Huelva, Alcalá del Río landfill in Seville and Cordoba landfill in Córdoba) are used as feeding habitat by *L. fuscus*, which connect these environments with wetlands used for roosting. Birds feeding at Río Tinto landfill usually roost in the Corumbel Bajo reservoir and Huelva Marshland, those at Alcalá del Río landfill roost at Gergal reservoir and in the Doñana ricefields, and those at Córdoba landfill usually roost at the Breña reservoir but sometimes at Fuente de Piedra lake (Martín-Vélez et al. 2020). Landfills provide unsuitable conditions for aquatic invertebrates and we saw no ponds or other wetlands on our visits there, so that propagules arriving to landfills are unlikely to establish.

Sample collection

Sampling for *L. fuscus* was conducted from November 2016 until March 2017, collecting a total of 414 samples including faeces (311) and pellets (103) (Table 1). In the ricefields, we also collected a total of 185 samples from *C. ciconia*, including 23 pellet and 51 faecal samples during November 2016, and 26 pellets and 85 faeces from September to November 2017 (Table 1). Fresh faeces and pellets were collected from roosting and foraging sites, where monospecific flocks were resting after feeding. Samples were taken from points separated by at least 1 m to ensure that they were from different individuals. To avoid contamination, we removed the surface in contact with the soil with a knife before storing the samples in separate zip bags. We stored the samples in the fridge at 4 °C until analysis (mean storage time = 35 days, range 4–80 days).

Sample processing

The fresh mass of pellet and faecal samples was first measured on a balance (Sartorius MSE225P) (Sartorious Lab Instruments, Goettingen, Germany). Samples were then sieved (100 µm mesh) and inspected under a stereomicroscope in Petri dishes. Invertebrate propagules [cladoceran ephippia, *Artemia* sp., other branchiopods, bryozoans (*Plumatella* spp.)] and snails were retrieved, counted, photographed, and measured (with ZEN 2–2.0 software) (Carl-Zeiss, Oberkochen, Germany). Seeds were also extracted at the same time (Martín-Vélez et al. 2021a). We only considered intact invertebrates or their propagules (henceforth referred to collectively as "invertebrates"), discarding broken ones, since our focus was on evidence for successful

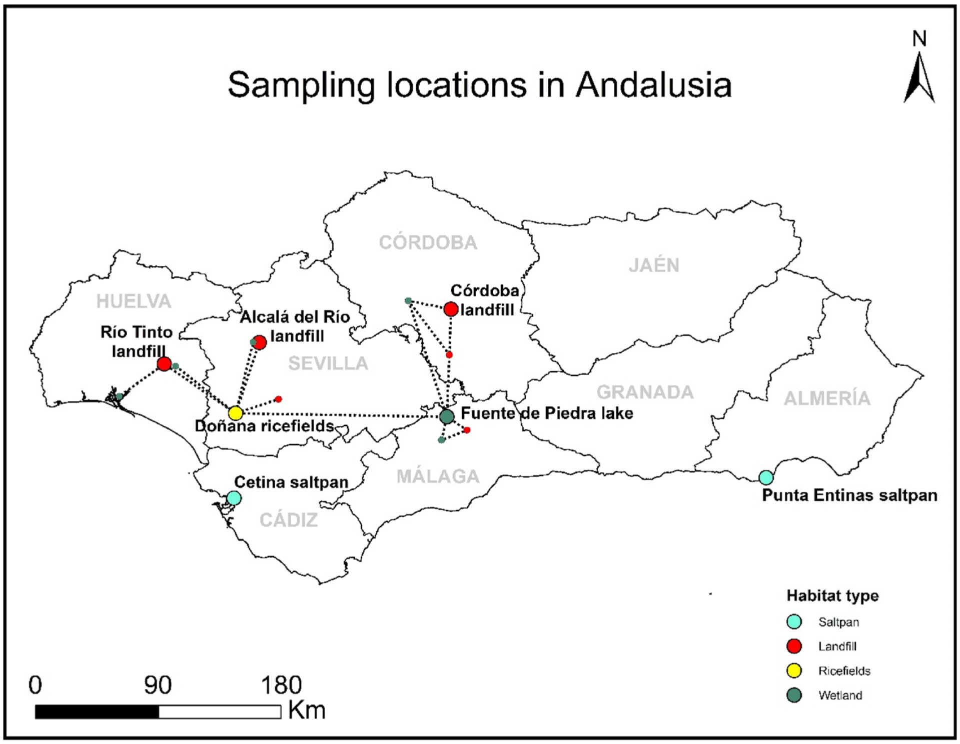

Fig. 1 Sampling locations in Andalusia, showing boundaries of the eight provinces. Big circles represent the sampling locations of this study, whereas small circles represent important unsampled habitats for connectivity based on Martín-Vélez et al. (2020). Dashed lines show connections between locations by direct flights. Habitat types are indicated by different colours (colour figure online)

dispersal. Bryozoan statoblasts were identified to species or genus level following Wood and Okamura (2005). Cladoceran ephippia were classified into 14 different morphotypes following criteria established by Vandekerkhove et al. (2004). Classification was based on their size, gross morphology, and special features from the digital pictures taken, such as number and position of the eggs. As with plant seeds (Costea et al. 2019), gut passage changes the morphology and coloration of invertebrate propagules. Therefore, because diagnostic features (position of spines and shape of the dorsal ridge) are no longer reliable after gut passage, in most cases, we were unable to assign species or genus to morphotypes with confidence.

Hatching experiments

We followed different hatching protocols depending on the taxonomic group: (1) *Artemia* sp., (2) cladoceran ephippia, (3) *Plumatella* sp., and (4) other branchiopods. After extracting *Artemia* cysts from samples (protocol 1), cysts were stored in the fridge at 4 °C in dry conditions for 4–7 days until hatching experiments began. Cysts were incubated in individualized glass petri dishes with filtered seawater (25 g/l with a pH of 8) under continuous illumination for 48 h, following Sánchez et al. (2007). After hatching, nauplii were transferred to 60 cm^3 vessels and fed with lyophilized algae *Tetraselmis chuii* until adults could be identified at a species level. Cladoceran ephippia (protocol 2) were stored for 107–177 days in dark conditions to break dormancy in water of 2% salinity (Ślusarczyk et al. 2019). *Plumatella* spp. and other branchiopods (protocol 3 and 4) were placed directly to hatch without prior storage. Ephippia, *Plumatella* spp. and other branchiopods were placed in plastic tubes in germination chambers with a 12/12 photoperiod and 22 °C/18 °C temperature conditions with water of 2 g/l salinity.

Data analyses

We carried out the most detailed analyses for samples from ricefields, because of the availability of two bird species for comparison, the multiple sampling periods, and two sample types (faeces and pellets). Abundance and richness (per sample) of macroinvertebrates were taken as the dependent variable, with sample type (faeces or pellets), species (gull or stork), and period (November 2016, September 2017, October 2017, and November 2017) as fixed factors, using sample weight as a continuous variable and sampling location as a random factor (see Martín-Vélez et al. (2021a) for details). We used Generalized Linear Mixed Models (GLMM) with negative binomial error distribution and logit link function under the *glmmTMB* package (Magnusson et al. 2017), to account for the many samples with zero values, and overdispersion.

We also used non-parametric statistics (Kruskal–Wallis and Dunn test) to compare the invertebrate abundance per sample across different sites in Andalusia for *Larus fuscus*. We tested differences in sample abundance for snails, statoblasts, ephippia, and other branchiopod eggs.

Results

Invertebrates dispersed by gulls and storks

A total of 642 intact invertebrates (including propagules) from seven different groups were recorded in excreta from gulls and storks (Tables 2 and 3). These groups represented bryozoa, cladocera, other branchiopoda, and gastropoda. Overall, 51.6% (239 of 464) of excreta samples (combining storks and gulls) contained at least one intact invertebrate (Table 2). Invertebrates were recorded in gull samples at all sites except Punta Entinas saltpans (Table 3). In ricefields, 35% of stork pellets and 67% of stork faecal samples contained at least one intact invertebrate, compared to 43% of gull pellets and 48% of gull faeces. Outside of ricefields, 23% of gull faeces contained at least one intact invertebrate.

Comparison between bird species in ricefields

The total abundance of intact invertebrates in samples was significantly related to bird species, sample type (pellet or faeces), and sample mass, with all variables having significant partial effects (Table 4). Similar results were recorded for invertebrate taxa richness (Table 4). Gulls and pellets had significantly fewer invertebrates, and fewer taxa per gram of excreta, than storks and faecal samples, respectively (Table 4). Neither abundance nor taxa richness was significantly influenced by sampling period (Table 4).

Comparison of gull excreta between sites

Differences in abundance of invertebrates between the gull samples (including faeces and pellets) at the seven sites were highly significant (Kruskal–Wallis test, $\chi^2 = 42.37$, $df = 5$ $p < 0.0001$). No intact propagules were recorded in the 40 samples processed from Punta Entinas saltpans, so this site was not included in this analysis or those below. Dunn post hoc tests showed that ricefields had significantly higher abundance per sample than all other sites. Other site combinations did not show significant differences. It is noteworthy that branchiopod eggs were recorded in all three landfill sites (Table 3).

Table 2 Details of intact invertebrates or their propagules found in *Larus fuscus* and *Ciconia ciconia* excreta samples from Doñana ricefields

Doñana Ricefields	*n* Samples	Cladoceran ephippia	Other branchiopod	*Plumatella fungosa*	*Plumatella repens*	*Plumatella sp.*	*Physella acuta*
Gull faeces	280	45/84 (15)	40/40 (5)	–	–	9/20 (5)	10/17 (3)
Gull pellets	98	17/22 (3)	10/12 (2)	1/1 (1)	–	7/7 (1)	8*/31 (18)
Stork faeces	137	38/126 (50)	55/108 (6)	–	1/1 (1)	28/33 (3)	2/2 (1)
Stork pellets	45	9/18 (7)*	8/13 (3)	–	–	2/3 (2)	4/5 (2)

Shown are total number of samples per site; number of samples each taxon was recorded in/total number of propagules (maximum number of propagules in a single sample)

*Cases where viability was confirmed (see main text). Underlined species are alien to the study area

Table 3 Details of intact invertebrates or their propagules found in *Larus fuscus* excreta samples from sampling sites across Andalusia

Site	*n* Samples	Cladoceran ephippia	*Artemia franciscana* cysts	Other Branchiopod egg	*Plumatella fungosa*	*Plumatella* sp.	*Physella acuta*
Alcalá del río landfill	19	–	–	1/1 (1)	–	–	–
Cordoba landfill	33	1/1 (1)	1/1 (1)	6/6 (1)	–	–	–
Fuente de Piedra	75	4/4 (1)	4/4 (1)	5/6 (2)	–	–	–
Cetina saltpan	30	1/1 (1)	8*/20 (11)	2/4 (2)	–	–	–
Rio tinto landfill	16	–	–	4/5 (2)	–	–	–
Doñana ricefields	370	62/106 (15)	–	62/70 (5)	1/1 (1)	16/27 (5)	18*/48 (18)

Punta Entinas saltpan was excluded as no invertebrate taxa were recorded. Shown are total number of samples per site; number of samples each taxon was recorded in/total number of propagules (maximum number of propagules in a single sample). Underlined taxa are alien to the study area

*Cases where viability was confirmed (see main text). Underlined species are alien to the study area

Table 4 Effects of bird species, period, sample type and weight on (A) total abundance of invertebrates and (B) taxon richness per sample from ricefields, from mixed models with a negative binomial error structure

(A) Propagule abundance	Level of effect	β	S.E	Z	p
Species	*Larus fuscus*	− 0.25449	0.12904	1.950	**0.0486**
Period	Sept. 2017	0.18130	0.17057	4.127	0.248
	Oct. 2017	− 0.06881	0.17994		
	Nov. 2017	0.02758	0.17476		
Sample mass		0.076	0.014	30.31	**< 0.001**
Sample type	Pellets	− 1.07374	0.20168	− 5.324	**< 0.001**
(B) Propagule richness	**Level of effect**	**β**	**S.E**	**χ2**	**p**
Species	*Larus fuscus*	− 0.23015	0.11287	− 2.039	**0.0414**
Period	Sep. 17	0.19718	0.15287	1.290	0.1971
	Oct. 17	− 0.06595	0.16043		
	Nov. 17	0.05734	0.14596		
Sample mass		0.07159	0.01179	6.073	**< 0.001**
Sample type	Pellets	− 0.91421	0.17392	− 5.256	**< 0.001**

Bold numbers represent significant values

Random contribution (variance): location = 0.003. Sampling location was included as a random factor. White stork, faecal samples, and November 2016 are absent from the table, because these levels of the respective factors were aliased, and so effectively had estimates of zero. Shown for each term are the parameter estimates (β) and their standard errors, and the main effects for each predictor variable

Bryozoan statoblasts and the alien snail *Physella acuta* were only recorded in ricefields, where they were significantly more abundant than in the other sites (Table 2 and 3, Table S1, S2). One of the 43 *Physella acuta* individuals was recorded alive when the pellet sample was observed under the binocular microscope 24 days after collecting it on 7th Nov 2016 and then storing it in the fridge (https://youtu.be/JzwwcUzh1Hs; Fig. S3). Given that none of the other snails were extracted quickly from the excreta samples, it is possible that many of them were alive at the time of collection.

Cladoceran ephippia were significantly more abundant in ricefields, but were also recorded in one landfill, one lake, and one saltpan complex (Table 3, Table S3). Ephippia were classified into 14 morphotypes (see Table S4, Fig. S1), and the most abundant (morphotype 1, $n = 80$) was provisionally identified as *Ceriodaphnia* cf. *quadrangula* (Table S4, Fig. S1.1). Eggs of other branchiopods (including *Artemia*) were significantly rarer at Alcala del Río landfill than at Cetina saltpans (Table 3, Table S5, Fig. S2). Branchiopod eggs (including *Artemia* and other large Branchiopods) were present in all sites (except Punta Entinas saltpan and Alcalá del Río landfill). Specifically, *Artemia* cysts were mainly found in Cetina saltpan and 66.7% (20 of 30) of the samples contained at least one cyst (Table 3).

Hatching experiments

64% (13 of 20) of the *Artemia* cysts hatched under laboratory conditions, taking on average 1.5 days to hatch and being identified upon maturity as the alien American brine shrimp *Artemia franciscana*. From the field season 2016–2017 in ricefields, 3 out of 36 ephippia (8%) hatched, and all three hatchlings were *Macrothrix rosea* (morphotype 4). None of 79 ephippia recorded during the sampling season 2017–2018 hatched. No propagules from other taxonomic groups (other branchiopod eggs and statoblasts, see Fig. S2) hatched.

Discussion

We identified the invertebrate taxa dispersed by gulls and storks through endozoochory within the most extensive ricefield area in Spain during the autumn migration and overwintering period. We also found spatial variation across a range of habitats in the invertebrates dispersed by gulls, with more abundance and diversity in ricefields. However, propagules were present in all habitats, even landfills visited by birds roosting in wetlands. In ricefields, storks and gulls dispersed similar invertebrates, although stork excreta had a higher density of dispersed organisms. Previous work (see "Introduction") has focused on endozoochory by Anatidae (ducks, geese, and swans) and charadriiformes (shorebirds), and our findings add to growing evidence that dispersal of invertebrates by migratory waterbirds is a ubiquitous ecological process. Furthermore, the gulls and storks are likely to have also been dispersing microbes such as rotifers and ciliates not quantified by our methods (Moreno et al. 2019; Silva et al. 2021).

Macroinvertebrate dispersal within ricefields

The dynamism and the semi-permanent, shallow, productive aquatic conditions of the ricefields provide a suitable habitat for many cosmopolitan species of macroinvertebrates with or without resistant propagules. As for plant seeds (Martín-Vélez et al. 2021a), the main pathway of invertebrate dispersal by *C. ciconia* and *L. fuscus* within and away from ricefields is likely to be secondary dispersal of propagules carried by alien crayfish (*P. clarkii*) ingested by the birds during the rice harvest (see also Lovas-Kiss et al. (2018)). Given the small size of the propagules recorded, it seems unlikely that gulls or storks would be actively foraging on them. In contrast, it is likely that the alien snails dispersed were large enough to be ingested deliberately as prey, just as waste rice grain is also consumed by gulls and storks (Martín-Vélez et al. 2021a).

Lovas-Kiss et al. (2018) previously reported three Cladoceran taxa in *L. fuscus* excreta from the Doñana ricefields, but not including *M. rosea* which we hatched from ephippia. Only *M. rosea* hatched in our study, and this is a cosmopolitan species common in ponds or lakes rich in organic matter (Huang et al. 2011). Seed viability has been shown to reduce with increasing storage time within the same study area (Martín-Vélez et al. 2021a). Similarly, storage procedures during our study may have reduced the rate of hatching of invertebrate propagules compared to natural conditions. Lovas-Kiss et al. (2018) did report *Ceriodaphnia* cf. *quadrangular*, which is likely to correspond to our most abundant morphotype 1 (Table S4). Amongst bryozoan statoblasts, we recorded both *Plumatella repens* and *P. fungosa*), and only the latter was reported by Lovas-Kiss et al. (2018). Therefore, apart from *M. rosea*, viable propagules of many other invertebrates (e.g., *Ceriodaphnia* cf. *quadrangular, Plumatella* spp.) are also likely to be dispersed by *L. fuscus* and *C. ciconia* under natural conditions in and beyond the ricefields.

After controlling for sample mass, invertebrate abundance and taxon richness were higher in stork excreta than in gulls, and higher in faeces than in pellets. This is consistent with the previous results for the same samples for seed abundance and richness (Martín-Vélez et al. 2021a). As with seeds (Martín-Vélez et al. 2021a), invertebrate propagules of a smaller size are more likely to pass through the intestines than to be regurgitated with larger undigested items. The causes of the difference between bird species are unknown, but could be related to differences in digestive efficiency, or prey ingestion as *C. ciconia* weighs about four times as much as *L. fuscus* and has a much larger gape. In contrast, *L. fuscus* is about ten times more abundant in the ricefields than *C. ciconia* (Rendón et al. 2008), so even if each individual stork disperses more propagules per day, overall the gull population is likely to disperse invertebrates in greater numbers. Furthermore, although both birds disperse similar invertebrate taxa (as observed for seeds, Martín-Vélez et al. 2021a), they have different movement patterns, so are likely to move invertebrates into different habitats. For example, when leaving ricefields gulls are more likely to fly to Fuente de Piedra lake (Fig. 1, and Martín-Vélez et al. 2021b), whereas storks are more likely to fly to the natural marshes of Doñana Natural Space (Ramo et al. 2013). Other waterbirds feeding in ricefields (e.g., ducks, shorebirds, flamingos, egrets, and ibis) are also likely to be important dispersal vectors for aquatic invertebrates. Multiple studies have now shown that resistant propagules of water fleas (freshwater cladocerans), anostracans (fairy and brine shrimps) and bryozoans (moss animals) are dispersed by a range of waterbirds through endozoochory (see "Introduction"), although to our knowledge, ours is the first study of storks.

The alien snail *P. acuta* is particularly widespread outside its native range in North America, and is considered the most cosmopolitan snail (Dillon et al. 2002; van Leeuwen et al. 2012b). An ability to disperse inside birds may help to explain its distribution and invasiveness. The dispersal mechanisms previously suggested for *P. acuta* include water (Van de Meutter et al. 2007), boats (Albrecht et al. 2009), and epizoochory on the feathers of waterbirds (Roscoe 1955), but endozoochory has not previously been considered. van Leeuwen et al. (2013) made a detailed study of *P. acuta* within the Doñana wetland complex including the ricefields (which was the only species found at the study area), and found high rates of gene flow between snail populations, as would be expected given our findings. Despite historical emphasis on epizoochory (Darwin 1872), snail endozoochory seems to be a more common mechanism than previously thought, as recent studies have demonstrated the ability of other snail taxa to survive gut passage through ducks or terrestrial birds (Cadee 2011; Wada et al. 2012; van Leeuwen et al. 2012b; Simonova et al. 2016). However, to our knowledge, our single observation of a live *P. acuta* is the first case of a snail surviving in pellets regurgitated by birds, indicating the possibility that snails may be dispersed in this manner as well as in faeces. It is possible that many other snails were alive when samples were collected, and hence, further work needs to be done to assess the real importance of waterbirds as vectors for *P. acuta* dispersal.

There is little previous information from field studies about how transport of viable invertebrates varies between excreta type (faeces vs pellets), and how this affects the viability of propagules and retention time, and consequently the quality of the dispersal (Martín-Vélez et al. 2021a). For example, propagules excreted in pellets may be expected to have increased likelihood of surviving as they avoid digestive processes within the intestines; alternatively, invertebrates expelled in pellets may be less viable, because they have been squeezed against hard prey items (Sánchez et al. 2005; Green et al. 2005). The type of excreta is also likely to have an important effect on the dispersal distance, which is related to gut retention time (Martín-Vélez et al. 2021a). Egestion in faeces is likely to provide a broader range of retention times and consequently dispersal distances (see Viana et al. 2013 for *Artemia* cysts), and faeces can be egested during flights or in feeding habitats, whereas pellets are more likely to concentrated in roosting habitats.

Endozoochory by gulls at other sites

In a study of the connectivity network between key sites for *L. fuscus* in Andalusia, the Doñana ricefields were the node with the highest centrality, i.e., with the highest number of connections across Andalusia (Martín-Vélez et al. 2020). Doñana ricefields, which its diverse invertebrate community of alien and native species, can be a good "source" site for dispersal of invertebrates to other habitats via birds. A modelling study taking the ricefields as source of seed dispersal by gulls estimated that seeds may reach dispersal distances beyond 150 km, allowing a gull to transport a propagule directly from ricefields to Fuente de Piedra lake (Fig. 1, and Martín-Vélez et al. 2021b). Direct flights from the ricefields are also regularly made to the landfills at Alcalá del Río, Córdoba and Rio Tinto (Fig. 1), and it is plausible that some of the propagules we recorded there were ingested in ricefields. The presence of ephippia and other branchiopod eggs in faecal samples from gulls in landfills is evidence of long-distance dispersal, since landfills are unlikely to be suitable for these invertebrates, and these propagules seem likely to have been ingested elsewhere in aquatic environments used for roosting or drinking. A series of landfills used for foraging are highly connected by gulls to different wetlands across Andalusia (Martín-Vélez et al. 2020); particularly, large numbers of *L. fuscus* roost at Fuente de Piedra lake during winter, a hypersaline shallow lake supporting various branchiopod taxa (Garcia et al. 1997). These gulls feed mainly in four different landfills in the surroundings, including Cordoba landfill (Fig. 1, and Martín-Vélez et al. 2019).

At Cetina saltpans, gull samples showed high abundance of viable cysts of the invasive North American brine shrimp *A. franciscana*. Endozoochory of *A. franciscana* cysts was previously demonstrated for shorebirds in Andalusia such as Redshank *Tringa totanus* or Dunlin *Calidris alpina* (Green et al. 2005; Sánchez et al. 2012). This is the first study to demonstrate endozoochory of this widespread alien by a gull, although other gull species are known to prey on brine shrimp, as indicated by the existence of cestode gull parasites using *Artemia* as an intermediate host (Sánchez et al. 2013).

Conclusions

This study builds on previous knowledge of the role of waterbirds as vectors of dispersal for aquatic invertebrates, providing the first information for storks, and the first study to document spatial variation in endozoochory rates in different nodes of a known connectivity network between habitats used by a migratory bird. *Larus fuscus* and *C. ciconia* are likely to facilitate effective dispersal and colonization of invertebrates between habitats through both pellets and faeces. Global change (e.g., changes in land-use) may increase the importance of avian endozoochory as a pathway for biological invasions. Extensive transformation in land use across Andalusia in recent decades includes reductions in the extent of natural wetlands, but increases in the surface area artificial wetlands such as ricefields, fish ponds, or irrigation ponds (Zorrilla-Miras et al. 2014). Our findings emphasize the potential that waterbirds have to enable invasive species to spread in their introduced range, although avian vectors are often overlooked by invasion biologists (Green 2016). Both alien molluscs such as the *P. acuta* and alien branchiopods such as *A. franciscana* are readily transported by waterbirds, especially those associated with anthropogenic, highly invaded environments such as ricefields and saltpans.

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Declarations

Conflict of interest Authors declare no conflict of interests.

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