

Transport of brine shrimps via the digestive system of migratory waders: dispersal probabilities depend on diet and season

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Abstract Waterbirds are known to disperse invertebrate propagules that survive gut passage, but there is very little information about how the probability of dispersal changes at different times of the annual cycle when birds move in different directions, or how it is affected by changes in diet. We studied internal transport of brine shrimp *Artemia* cysts by migratory waders in the Odiel saltworks in south-west Spain. Viable cysts of parthenogenetic *Artemia* were abundant in the faeces and regurgitated pellets of redshank *Tringa totanus*, pellets of spotted redshank *T. erythropus*, and faeces of black-tailed godwit *Limosa limosa* during spring and/or autumn migrations in 2001–2002, but were not recorded during winter. Godwits did not produce pel-

lets, and spotted redshank faeces were not sampled. Significant correlations between the number of cysts in a pellet or faecal sample and the proportion of that sample constituted by *Artemia* adults suggested that most cysts were ingested while in the ovisacs of gravid females. The proportion of cysts destroyed during digestion increased when accompanied by harder food items or grit, and when fewer cysts were ingested. The median number of intact cysts was higher in redshank faeces than in their pellets, but cysts extracted from pellets were more likely to hatch. A higher proportion of redshank pellets contained *Artemia* cysts in spring than in autumn, but more redshank migrated through the area in autumn. Significantly fewer cysts were recorded in redshank pellets in winter than in spring or autumn. Our results confirm that there is potential for long-distance dispersal of *Artemia* cysts via waders during both northwards (spring) and southwards (autumn) migrations.

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Introduction

Darwin (1859) proposed that migratory waterbirds played a major role in the dispersal of aquatic invertebrates. Waders (Charadriiformes) are likely to be particularly good vectors owing to their long-distance migrations (del Hoyo et al. 1996). However, the importance of birds compared with other means of dispersal such as wind or human activity remains unclear (Havel and Shurin 2004; Green and Figuerola 2005). Although laboratory studies have shown that propagules of various invertebrates can survive passage through the avian digestive system (Proctor 1964; Proctor et al. 1967; Figuerola and Green 2002a; Green and Figuerola

2005), there remains a shortage of studies demonstrating that such dispersal occurs in the field (Proctor 1964; Bohonak and Jenkins 2003; Figuerola et al. 2003). In the most complete field study of passive internal transport to date, Green et al. (2005) showed that viable brine shrimp *Artemia* (Anostraca) cysts (resistant encysted embryos) are excreted in faeces and pellets by waders on autumn migration. As well as excreting faeces, some waders excrete pellets from the gizzard by regurgitation (Sánchez et al. 2005).

There is currently no information from field studies about how transport of viable invertebrate propagules varies from one part of the year to another, or with the diet of an individual bird. Such information is vital to our understanding of the role of birds in the ecology and genetics of invertebrate populations. For example, dispersal in one direction during spring migration has different consequences for colonization and genetic exchange between populations than dispersal in the opposite direction during autumn migration. Dispersal as birds move towards higher latitudes in spring migration is likely to be especially important to facilitate changes in distribution of invertebrate taxa in the face of global warming. The timing of dispersal is also likely to have a profound effect on the chances of establishment when a propagule reaches a new environment (de Meester et al. 2002). Transport rates could be expected to vary seasonally given the considerable temporal variation in waterbird diets (Green et al. 2002) and in the production of invertebrate propagules (Wurtsbaugh et al. 2001). In this study we test the hypothesis that levels of dispersal of *Artemia* cysts by different wader species migrating through the Odiel marshes in southwest Spain vary at different times of the year. We study redshank *Tringa totanus* excreta from three seasons, and spotted redshank *T. erythropus* and black-tailed godwit *Limosa limosa* excreta from one season each. We quantify numbers of intact propagules, assess their viability and compare the potential for dispersal during spring and autumn migrations. Switching waterbird diet in the laboratory changes the proportion of cysts surviving gut passage (Charalambidou et al. 2005). We test the hypothesis that wader diet determines two components of the dispersal process: rates of ingestion of cysts and the likelihood of cysts surviving the digestive process. Since propagules excreted in pellets avoid passing through the hostile environment in the intestines, they may be expected to survive more readily than those that are defecated. We compare the abundance and hatchability of cysts excreted in pellets and faeces by redshank, and consider the consequences for dispersal.

Materials and methods

Sample collection

A total of 140 faecal samples and 220 pellets were collected in 2001 and 2002 from waders in the Aragonesas-Bacuta saltworks of the Odiel marshes in Huelva province (37°17'N 06°55'W, area 7,158 ha), a site of international importance for migratory waders migrating through the East Atlantic flyway (Sánchez et al. 2006a; Stroud et al. 2004). Given the numbers of birds present (see below), it is highly likely that each sample was from a different bird. For the purpose of seasonal comparison, sampling dates from March to April were considered as spring, July to August as summer, September to October as autumn and January to February as winter. Spring samples coincided with the beginning of the northwards wader migration, and summer and autumn samples with the southwards migration (Sánchez et al. 2006a, b). Pellets and faeces were collected from redshank, pellets from spotted redshank, and faeces from the black-tailed godwit (a species not known to regurgitate pellets). These species were selected because of their abundance in the study area and because their droppings and pellets are large, helping to avoid soil contamination. Redshank and black-tailed godwit are two of the four most numerous waders at this site (Sánchez et al. 2006a). In our study area in 2001, numbers of redshank peaked at 2,170 birds, godwits at 6,684 and spotted redshank at 351 (see Sánchez et al. 2006b for details).

We collected fresh faeces and pellets from roost sites on dykes in saltworks used by monospecific flocks at high tide. The great majority of birds roosted in the water or in multispecific flocks, making sample collection necessarily opportunistic. Combined with the different temporal patterns of abundance and migration between species, this made it impossible to sample the same species on the same dates. Thus redshanks were sampled in spring, autumn and winter, spotted redshank only in spring, and godwits only in summer.

Sample collection points were observed for 30 min prior to sampling and only fresh samples were taken to ensure a match with bird species. Samples taken were separated by at least 1 m from each other to avoid taking multiple samples from the same bird. Each sample of excreta was carefully separated from the soil (discarding that part in contact with soil) and placed in a tube. Each excreta sample contained only a small fraction (roughly 10%) of the cysts excreted in a 24 h period by a given bird (M.I. Sánchez, F. Hortas, J. Figuerola and A.J. Green, unpublished data).

The Aragonesas-Bacuta saltworks contain 60 individual salt ponds, most of which hold *Artemia* populations. Waders move continuously between individual ponds, and continuous monitoring of birds in the study area showed that most of the birds we sampled had not been feeding in immediately adjacent ponds prior to roosting on dykes. Thus most *Artemia* cysts present in faeces had been transported from different parts of the saltworks or from further away.

Sample processing

The samples were stored at 5°C in the dark, until the time of analysis (a period of 6–12 months). Each sample was then washed on a 0.04 mm sieve, and intact *Artemia* cysts were extracted by flotation in hypersaline brine, counted, then washed in distilled water. They were then dried for 48 h at 40°C, followed by storage at 5°C in the dark prior to hatching experiments. The composition of the rest of the faecal and pellet samples was also identified, counting broken cysts and assessing the proportion of sample volume made up of different food items or grit using the following categories: 0%, <10%, 11–25%, 26–50%, 51–75%, 76–90% and >90% of total volume (see Sánchez et al. 2005 for details). Broken cysts were easy to count, as the outer case remained in one piece or was split down the middle into two pieces.

Some samples were collected with the principal aim of studying wader diet (Sánchez et al. 2005) and were stored in alcohol. Thus, hatching was tested for a subset of samples. Cysts were incubated in diluted filtered sea water (25 g/l with a pH of 8) at 26°C and under continuous illumination for at least 48 h. Hatched nauplii were counted and transferred into 60 cm³ vessels and cultured in 70 g/l filtered brine (seawater plus crude sea salt), on a diet of live *Dunaliella salina* and *Tetraselmis suecica* microalgae. They were maintained at 24°C, under aeration on a 12D:12L photoperiod. The medium was monitored and renewed every 2 days. Resulting adults were identified to species morphologically (after Amat et al. 2005). Survival to adulthood was 68%.

Statistical analysis

Numbers of intact cysts in excreta

We attempted parametric analyses of the numbers of intact cysts in samples in relation to differences between season, wader species or sample type, using a range of error distributions and transformations. However, owing to the high proportion of zeros in the data and severe problems of over-dispersion and model convergence, we were unable to produce satisfactory

models. We therefore used non-parametric Kruskal–Wallis and Mann–Whitney *U*-tests employing Statistica 5.5 (StatSoft 1999). Significant results for pairwise post-hoc tests following a Kruskal–Wallis test were maintained after correction for multiple testing (Rice 1989).

Artemia cysts ingested by waders can potentially be those present in the sediments, those in the water column, those forming floating masses on the surface or those situated in the ovisacs of adult females (Green et al. 2005). Only in the last case, a significant correlation would be expected between the abundance of adult *Artemia* and of cysts in different samples. Following their release from the ovisac, the distribution of cysts and adult *Artemia* within a given salt pan is very different (Sánchez et al. 2006c), such that birds feeding on adults would not be more likely to ingest released cysts in an incidental manner. We calculated Spearman rank correlations between the abundance of *Artemia* adults (according to the seven volumetric categories defined in the previous section on sample processing) and the total number of cysts recorded in redshank and godwit samples.

Survival and hatchability of cysts

We used generalized linear models (GLMs) to analyze simultaneously the effects of season, wader species (redshank or godwit), sample type (faeces or pellet) and diet on the percentage of cysts in a sample that were broken, and the percentage of intact cysts that hatched. Predictor variables were included as fixed factors. Season was included as a factor of three levels (excluding winter because no cysts were recorded) and both sample type and wader species as factors of two levels. Diet was included as a factor of three levels according to whether the majority of the sample was made up of soft items (e.g. chironomid larvae or *Artemia*), hard items (e.g. bivalve shells or grit) or items of intermediate hardness (e.g. Coleoptera, Ephydriidae larvae or polychaetes). Because of their small sample size, data for spotted redshank were excluded from the models. Since godwits were only sampled in summer and redshank only in other seasons, and season was included in the models as a factor, we could not test the partial effect of wader species on the percentage of broken or hatching cysts.

We carried out the GLMs following the GENMOD procedure in SAS (Version 8.2, SAS Institute 2000) using a normal error distribution and identity link, and arcsine transformed the dependent variable to overcome heteroscedasticity. Attempts to analyze the dependent variables with a binomial error in a logistic regression (Crawley 1993) were abandoned because of severe over-dispersion. In order to test for a relationship

between the proportion of cysts broken or hatching and the total number of cysts in a sample, we conducted GLMs in which the total number of cysts (log transformed) was included as an additional predictor variable. The significance of the reduction in deviance (equivalent to a reduction in variance) for the models of cyst breakage or hatching was derived from *F*-tests, scaled to control for over-dispersion (Crawley 1993).

Results

Numbers of intact cysts in excreta

Intact *Artemia* cysts were recorded in the droppings and/or pellets of all three wader species and in all seasons except winter (Table 1). Cysts were most abundant in godwit faeces in summer 2001, and were also very abundant in redshank samples in spring and autumn (Table 1, Fig. 1). Overall, 85.3% of cysts (*n* = 57729) recorded in samples were intact.

For redshank pellets, there were highly significant differences between seasons in the number of intact cysts per sample (Kruskall–Wallis test, *n* = 211, *H* = 44.16, *df* = 2, *P* < 0.0001). Post-hoc tests showed that the number of intact cysts in spring (median = 3) was significantly higher than in autumn (median = 0, Mann–Whitney *U*-test, *U* = 1247, *P* < 0.0001) and in winter (median = 0, *U* = 94.5, *P* < 0.0001). Likewise, the number of intact cysts was higher in autumn than in winter (*U* = 1302, *P* = 0.021). The main difference between spring and autumn was that a higher proportion of pellets contained intact cysts in spring (Fig. 1a,

$\chi^2_1 = 34.7$, *P* < 0.0001), rather than a difference in the number of cysts in those pellets. For redshank pellet samples with at least one intact cyst, there was no significant difference in the number of cysts between spring (median = 5.5) and autumn (median = 3, *U* = 392.0, *P* = 0.95). In contrast to the result for medians, the presence of up to 15,445 cysts in a single pellet caused the mean number of intact cysts per pellet for autumn to be higher than for spring (Table 1).

Amongst redshank samples from autumn, the median number of intact cysts was significantly higher in faecal samples than in pellets (*U* = 4997, *P* = 0.0001). In contrast, the maximum and mean were higher in pellets (Table 1). Amongst spring pellets, numbers of intact cysts per sample transported by redshank were higher than those transported by spotted redshank (Fig. 1), but the difference was only marginally significant (*U* = 85.00, *P* = 0.051). Intact cysts were present in a significantly higher proportion of godwit faeces samples in summer 2001 than summer 2002 (Table 1, Fig. 1b, $\chi^2_1 = 31.8$, *P* < 0.0001).

For both faecal and pellet samples, there was a strong relationship between the total number of cysts recorded in each sample and the proportion of that sample made up by *Artemia* adults (godwit faeces, *r_s* = 0.783, *P* < 0.0001, *n* = 56; redshank faeces, *r_s* = 0.424, *P* < 0.0001, *n* = 84; redshank pellets, *r_s* = 0.237, *P* < 0.0001, *n* = 211).

Survival of cysts

There were no significant partial effects of season or sample type (pellet or faeces) on the percentage of

Table 1 Number of samples with *Artemia* cysts, number of intact cysts per sample and percentage of cysts that were broken

Bird species			Number of samples			Number of intact cysts per sample		Broken cysts (%)	
			Total	With cysts	With intact cysts	Mean ± SE	Range	Mean ± SE	Range
Redshank <i>Tringa totanus</i>	Faeces	Aut-01	84	51	38	98.1 ± 49.4	(0, 3339)	41.2 ± 5.5	(0, 100)
		Pellet	33	25	24	50.7 ± 36.3	(0, 1187)	14.8 ± 5.9	(0, 100)
		Aut-01	157	48	33	189.3 ± 105.0 ^a	(0, 15445 ^a)	41.1 ± 6.3	(0, 100)
		Win-01	21	0	0	0 ± 0	(0, 0)	–	–
Black-tailed godwit <i>Limosa limosa</i>	Faeces	Sum-01	42	41	41	221.6 ± 35.4	(0, 980)	0	(0, 0)
		Sum-02	14	5	3	0.3 ± 0.2	(0, 2)	40 ± 24.5	(0, 100)
Spotted redshank <i>Tringa erythropus</i>	Pellet	Spr-01	9	5	3	31.4 ± 31.1	(0, 280)	40 ± 24.5	(0, 100)
Total			360	175	142	136.8 ± 47.6	(0, 15445)	27.7 ± 3.0	(0, 100)

Spring represents northwards migration and summer and autumn southwards migration

^a Removing the sample with 15,445 cysts, mean ± SE = 91.6 ± 38.4, maximum = 3619, SE standard error

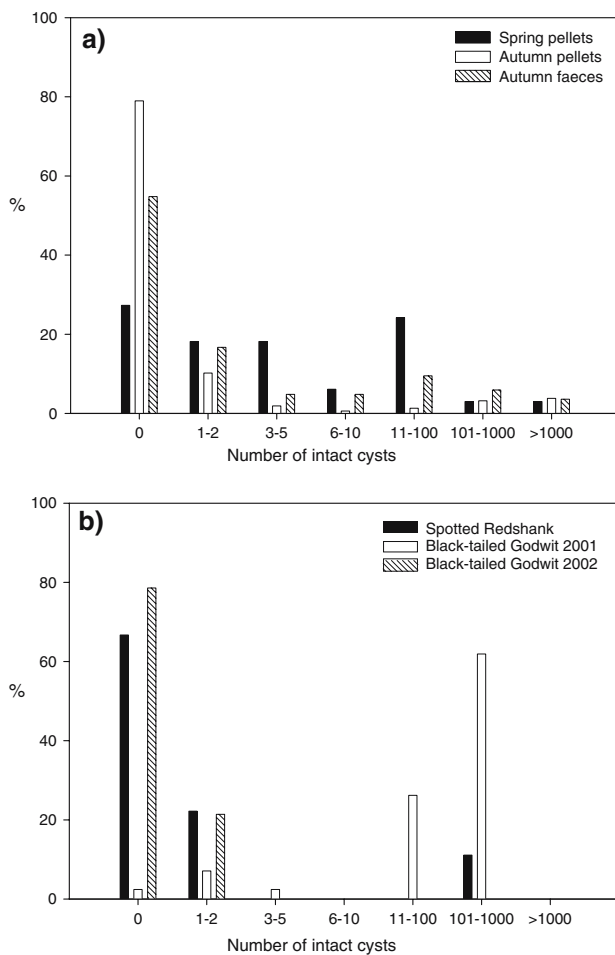


Fig. 1 Percentage of samples holding different numbers of intact *Artemia* cysts for (a) redshank *Tringa totanus* samples collected in different seasons and (b) spotted redshank *T. erythropus* and black-tailed godwit *Limosa limosa*. See Table 1 for sample sizes and details of winter redshank pellets

cysts that were destroyed during gut passage (Table 2). However, diet had a highly significant partial effect, the percentage of cysts destroyed increasing with the hardness of other food items or grit in the sample (Table 2). With the percentage of cysts destroyed as the dependent variable and wader species, season, sample type (pellet or faeces) and total number of cysts as predictor variables, there was a significant negative partial correlation between the number of cysts and the percentage destroyed ($F_{1,169} = 19.12$, $r = -0.35$, $P < 0.0001$).

Hatchability of cysts

Viability was confirmed by hatching for cysts from samples from all three wader species, and for samples collected in spring, summer and autumn (Table 3). A total of 304 adult *Artemia* were reared from 446 nauplii and all were identified as diploid female parthenogens

based on morphometry (i.e. members of a polyphyletic group of parthenogenetic strains sometimes classified under the binomen *A. parthenogenetica*; see Abatzopoulos et al. 2002; Amat et al. 2005; Baxeveanis et al. 2006). Hatchability (measured as % of cysts hatching) was highest for redshank pellets in autumn (Table 3). There were no significant differences between seasons in the percentage of intact cysts hatching, but there was a significant effect of sample type, with a higher percentage of cysts hatching in redshank pellets than in droppings (Table 2). The partial effect of sample type remained significant when diet was added to a model including wader species and season ($F_{1, 65} = 4.84$, $P = 0.031$), but diet had no influence on the percentage of cysts hatching ($F_{2, 65} = 0.79$, $P = 0.46$).

With the percentage of intact cysts hatching as the dependent variable and species, season, sample type and total number of cysts as predictor variables, there was no significant correlation between the number of cysts and the percentage hatching ($F_{1, 67} = 0.83$, $r = -0.11$, $P = 0.37$).

Discussion

We have found black-tailed godwits, redshank and spotted redshank to be effective dispersers of parthenogenetic *Artemia* at the Odiel marshes, where thousands of waders pass through on migration between northern Europe and Africa (Wetlands International 2002; Stroud et al. 2004). The East Atlantic Flyway, used by an estimated 15.5 million waders (Stroud et al. 2004), is likely to be a major route for long-distance dispersal of brine shrimps and other invertebrates. *Artemia* cysts are also likely to be transported by other migratory waterbirds using salt pans (Green et al. 2002), both internally and externally on the feathers and feet of waterbirds (Figuerola and Green 2002b). Inspection of waders trapped for ringing suggests they carry cysts externally, but in much smaller numbers than those recorded in excreta (J. Figuerola, A.J. Green, F. Hortas and M.I. Sánchez unpublished data). How far cysts can be transported externally depends on how long they stay attached, which is unknown.

The birds we studied included a mixture of birds that had just completed or were just about to commence long-distance movements, and birds that were making movements between feeding and roosting sites within our study sites. Transport of cysts by waders between salt ponds is likely to occur at different scales, with transport between different ponds within a complex being extremely frequent events. Dispersal events between different saltworks within the same estuary

Table 2 Generalized linear models of the partial effects of season (factor of three levels), sample type (factor of two levels) and diet (factor of three levels) on the percentage of *Artemia* cysts that were broken ($n = 170$ samples) and the percentage of intact cysts that hatched ($n = 71$ samples) in samples from godwits and redshank

	Effect	Estimate	SE	df_N	df_D	F	P
Broken cysts (%) $D = 27.23$	Constant	0.43	0.13				
	Season			1	164	0.45	0.5018
	Autumn	0.19	0.12				
	Spring	0.10	0.17				
	Sample type			1	164	0.83	0.3648
	Pellet	-0.10	0.11				
	Diet			2	164	13.29	<0.0001
Soft	-0.38	0.11					
Hard	0.35	0.12					
Cysts hatching (%) $D = 10.00$	Constant	0.07	0.04				
	Season			1	67	1.94	0.1681
	Autumn	-0.03	0.08				
	Spring	-0.31	0.22				
	Sample type			1	67	5.50	0.022
Pellet	0.24	0.10					

The dependent variables (left hand column) were arcsine transformed, and an identity link and normal error distribution were used. Wader species (factor of two levels) was also included in the models, but its partial effect could not be measured because each species was studied in different seasons. Spring and autumn samples were from redshank, summer samples from godwit. The partial effect for season represents the difference between redshank pellets in spring and autumn. The partial effect for sample type represents the difference between redshank pellets and faeces in autumn. Season = summer, sample type = faeces, and diet = medium hardness were aliased (i.e. estimate taken as zero)

D percentage of deviance explained by the final model compared to the null model, SE standard error of estimate, df_N degrees of freedom in the numerator for the F value, df_D degrees of freedom in the denominator for the F value

system (e.g. between the Aragonesas-Bacuta and Astur saltworks in the Odiel estuary, separated by 5 km) will occur with intermediate frequency. Dispersal between entirely different catchments will occur with lower frequency. For example, birds are known to move between the Odiel saltworks and those in the Guadalquivir estuary, separated by 65 km. Precise data on wader movements are required before these contrasting dispersal rates can be quantified.

The average time spent by waders at stopover sites during migration varies from one to 50 days and is unknown for sites in south-west Iberia. Godwits and other waders regularly move between salt pans and other wetlands separated by up to 20 km while at stopover sites (Farmer and Parent 1997, P.M. Potts, pers. comm. 2004), thus facilitating propagule dispersal between different parts of a wetland complex. Most major passage and wintering sites on the East Atlantic Flyway contain saltworks, facilitating the long-distance dispersal of *Artemia*. Waders on migration move rapidly between coastal wetlands, and distances between stopovers can easily exceed 1,000 km (Iverson et al. 1996; Pennycuik and Battley 2003). The modal retention time (much less than the mean) of viable *Artemia* cysts within another wader, the killdeer *Charadrius vociferus*, was 90 min, and the maximum was 26 h (Proctor et al. 1967). Redshank and godwits fly at

56–60 km/h (Welham 1994) suggesting that, during migration, the maximum dispersal distance of viable cysts is likely to be ca.1,500 km. However, the vast majority of cysts will be dispersed over distances of less than 100 km. The data on retention time by killdeer (Proctor et al. 1967; de Vlaming and Proctor 1968) suggests that, during migration, approximately 50% of viable cysts would be voided within 2 h or 120 km, a further 45% within 2–10 h or 120–600 km, and the final 5% within 10–26 h or 600–1,500 km.

Although rates of transport of cysts between neighbouring ponds via birds are very high, it is unclear whether or not they will hatch and become established, and what influence they may have on gene flow between *Artemia* populations. Active saltworks such as those studied have strong salinity gradients with major differences in salinity between ponds (Britton and Johnson 1987; Sánchez et al. 2006c), and fewer *Artemia* genotypes may tolerate the most extreme salinities (Browne and Hoopes 1990). Furthermore, casual observation shows that cysts can disperse between adjacent ponds in foam that forms at a pond edge and is carried on the wind (authors, unpublished observations).

Likewise, it is currently unclear whether *Artemia* transported by birds as cysts to other saltworks have a good chance of becoming successfully established.

Table 3 Number of *Artemia* nauplii hatched and the percentage of cysts that hatched

		Number of samples			Number of nauplii per sample ^a		Cysts hatching (%)	
		Total	With intact cysts	With nauplii	Mean ± SE	Range	Mean ± SE	Range
Redshank								
Faeces	Aut-01	58	14	3	0.4 ± 0.2	(0, 3)	3.9 ± 3.5	(0, 50)
Pellet	Spr-01	5	2	0	0.0	(0, 0)	0.0	(0, 0)
	Aut-01	133	13	6	3.0 ± 1.6	(0, 16)	19.2 ± 10.1	(0, 100)
Black-tailed godwit								
Faeces	Sum-01	42	41	26	9.7 ± 2.3	(0, 70)	7.4 ± 1.6	(0, 40)
Spotted redshank								
Pellet	Spr-01	8	2	1	2.0 ± 2.0	(0, 4)	2.3 ± 2.3	(0, 4.6)
Total		282	72	36	6.2 ± 1.4	(0, 70)	8.5 ± 2.2	(0, 100)

Sample sizes are lower than in Table 1 because not all samples were stored in such a way as to conserve viable cysts

SE standard error

^a For samples with at least one intact cyst

Parthenogenesis is likely to be advantageous for the colonization of new habitats not previously occupied by brine shrimps, since a single individual could potentially found a new population. It has been suggested that sexual species such as *A. salina* or *A. franciscana* should show greater local adaptation than asexual species, and thus that transport of propagules to established populations of the former are less likely to translate into measurable genetic exchange, owing to a monopolisation of the habitat by the locally adapted genotypes (de Meester et al. 2002; but see Green and Figuerola 2005). In other words, a given amount of long-distance transport of cysts by birds may result in greater interchange of genotypes for parthenogens than for sexual *Artemia*. Genetic data for asexual *Artemia* and *A. salina* in the Western Mediterranean support this prediction (J. Muñoz, A.J. Green, J. Figuerola, F. Amat and C. Rico submitted).

In a previous study at Cadiz Bay and Castro Marim in the Iberian peninsula during autumn migration, redshank were shown to disperse parthenogenetic *Artemia*, and black-tailed godwits and redshank were both shown to be effective dispersers of the alien *A. franciscana* (Green et al. 2005). Although the number of *Artemia* cysts per sample was higher at Odiel, their viability was lower than at Cadiz Bay and Castro Marim. This may be a consequence of the longer storage time of our samples between collection in the field and hatching (up to 15 months) which may have reduced viability. A notable contradiction between these two studies is that Green et al. (2005) found the percentage of cysts hatching to be higher for redshank faeces than for pellets, whereas the opposite was recorded in the present study. Both results were weakly significant and may be due to Type I errors, or else they may be due to the noise and variation in space

and time that can be observed when studying dispersal in the field. Cysts in pellets do not necessarily have the same origin as those in faeces, and this may potentially influence differences in viability between samples. For example, cysts consumed within female *Artemia* and those ingested separately may be processed differently. The former are likely to be ingested in different development stages, and immature cysts with an incomplete chorion are more prone to be digested. Some studies in captivity (Horne 1966; Charalambidou et al. 2005) suggest that gut passage itself does not affect the viability of those *A. franciscana* cysts that survive intact, yet their viability was reduced by gut passage in greater flamingos *Phoenicopterus ruber* when they were mixed with sand (MacDonald 1980).

As previously recorded by Green et al. (2005), we found that *Artemia* cysts were more abundant in redshank faeces than in pellets. This is likely to further long distance dispersal, as propagules are likely to be retained longer and transported further when expelled in faeces (Nogales et al. 2001). Redshank have also been found to excrete more *Mesembryanthemum nodiflorum* seeds in faeces than pellets, whilst seeds in pellets are more likely to germinate (Sánchez et al. 2006b). We expect that pellet-producing birds will also tend to excrete other invertebrate propagules in faeces, as this seems to be the general way they process smaller items (Sánchez et al. 2005).

Whereas the mode of ingestion of *Artemia* cysts was previously unclear (Green et al. 2005), we found strong evidence that many cysts recorded in faeces or pellets were ingested within the ovisacs of adult female brine shrimps. We often observed cysts within partially digested adults in faecal samples and pellets. The absence of cysts from winter pellets is consistent with such a pattern, as adults were absent from the water

column then. In the Odiel salt pans, cyst production peaks in spring and summer and *Artemia* adults almost disappear in winter, when cysts remain reasonably abundant in the water column and in the sediments (Sánchez et al. 2006c). Among 200 adult *Artemia* collected in the Odiel salt pans in August 2005, 97 had cysts visible in their ovisac (mean \pm SE = 19.3 ± 0.94 cysts per adult, range 1–53). However, the presence of occasional samples with very large numbers of cysts (Table 1, Fig. 1a) suggests that waders may also feed directly on floating concentrations of cysts often observed at the downwind edge of salt pans.

It has previously been suggested that dietary variation is likely to lead to differences between birds in survival rates of propagules (e.g. Figuerola and Green 2002a), but we are unaware of any previous field studies showing that diet influences the survival rate of ingested invertebrate propagules. We found that, for a given wader species, *Artemia* cyst survival was directly related to the hardness of food items. We also found that, as the number of cysts in a sample increased, the proportion that were destroyed decreased. This indicates that individuals which consumed more cysts also expelled a higher proportion of viable cysts. Such a relationship has also been reported for seed dispersal by waterfowl (Figuerola et al. 2002). We expect that the effects of the hardness of avian diet and propagule intake rates observed on propagule survival will be applicable to the dispersal of other aquatic invertebrates by waterbirds.

Major differences between autumn and spring migration in the probability of long-distance transport by waterbirds have been predicted for seeds (Clausen et al. 2002; but see Figuerola et al. 2003) and redshank at Odiel transported more seeds in spring than in autumn (Sánchez et al. 2006b). We have not found evidence for major differences in the dispersal rates of *Artemia* when waders migrate north or south. Godwit faecal samples from the beginning of the autumn (southwards) migration period in 2001 had the most cysts, but we obtained no godwit samples during spring migration. The comparison of transport rates for different seasons for a given wader species is complicated by the difficulties of collecting samples (see Methods). Whilst cysts were present in a higher proportion of redshank pellets in spring (76%) than in autumn (30%), there were more redshank and godwits passing through Odiel on autumn migration (Sánchez et al. 2006a, b). In 2001, *Artemia* abundance in the study area peaked from July to September (Sánchez et al. 2006c) during autumn migration, suggesting that seasonal variation in the rates of cyst transport by redshank are not a simple consequence of variation in the

availability of *Artemia* prey. In any case, data from one annual cycle are insufficient to identify a general difference in dispersal potential between spring and autumn. Stopover times and the probabilities that waders move between suitable habitats for the dispersed species are likely to differ between spring and autumn migration, and to vary between wader species, but there are currently no data on these important issues. Furthermore, dunlin *Calidris alpina* and little stint *C. minuta* are much more abundant at Odiel during spring than autumn migration (authors, unpublished data), and it is possible that these species are also dispersers of *Artemia*.

We expect that temporal and spatial variation in the abundance and distribution of invertebrates and their propagules, and in the abundance, distribution and diet of their avian vectors in aquatic systems will produce huge variation in dispersal rates and prevent them from being consistent between sites and years. One example is the striking difference between the summers of 2001 and 2002 in the numbers of *Artemia* cysts dispersed in godwit faeces (Fig. 1).

In summary, waders are major vectors of *Artemia* during both northwards and southwards migrations, with dispersal probabilities depending partly on diet and seasonal variation. More data are required on the role of wader species not included in this study, and on the probabilities that propagules are dispersed to suitable habitats (see Green and Figuerola 2005 for further discussion). Waders are likely to transport other invertebrates such as Chironomidae (Green and Sánchez 2006), Cladocera (Dodson and Egger 1980), horseshoe crabs (Castro and Myers 1993) or foraminifera (Wolf 2001). More detailed studies are required before the true significance of this dispersal can be established and compared to that of other vectors such as wind, water or man.

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