Comparing the potential for dispersal via waterbirds of a native and an invasive brine shrimp

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SUMMARY

1. Migratory waterbirds are likely to have a major role in the spread of many exotic aquatic invertebrates by passive dispersal. However, in the field, this has so far only been confirmed in the case of the American brine shrimp *Artemia franciscana*, which is spreading quickly around the Mediterranean region.
2. We compared experimentally the capacity of *A. franciscana* and the native brine shrimp *Artemia parthenogenetica* to disperse via migratory shorebirds. After *Artemia* resting eggs (cysts) were fed to Redshank *Tringa totanus* and Dunlin *Calidris alpina*, we compared the proportion that survived gut passage, their hatchability and their retention time within the gut. We also tested the ability of cysts to stick to the feathers of Black-tailed Godwit *Limosa limosa*.
3. The proportion of ingested cysts retrieved from faeces was the same for each *Artemia* species (8%), and there were no significant differences in retention time (mean 1.2 h and maximum 10 h for *A. parthenogenetica*, 1.4 and 12 h for *A. franciscana*) or hatchability (11% versus 14%). The two shorebird species showed similar retention times and retrieval rates, but cysts recovered from Dunlin had a significantly higher hatchability. Only one of the 1000 *A. parthenogenetica* cysts and three of the 1000 *A. franciscana* cysts stuck to feathers.
4. These results indicate that both non-native and native brine shrimps have a similar high capacity for endozoochory via birds, and that the invasiveness of *A. franciscana* is probably explained by its competitive superiority owing to high fecundity and release from cestode parasitism. Owing to their different migratory behaviour, Redshank and Dunlin are likely to have different roles as brine shrimp vectors. Brine shrimps provide a suitable model for understanding the role of birds in the dispersal of exotic aquatic invertebrates.

Keywords: *Artemia franciscana*, dispersal ability, Endozoochory, Epizoochory, retention time

Introduction

Exotic invertebrates are spreading across the world's aquatic ecosystems, with major economic consequences and impacts on indigenous biodiversity (Strayer, 2010). It has been argued that passive dispersal by migratory waterbirds plays an important role in the spread of invasive invertebrates, especially those with resistant propagules that can be transported externally or within the avian gut (Figueroa & Green, 2002a; Green & Figueroa, 2005). Similarly, a broad range of exotic plants are known to be dispersed by waterbirds (Green et al., 2008; Brochet et al., 2009). However, to date, such

transport of exotic invertebrates in the field has been demonstrated only for the American brine shrimp *Artemia franciscana* Kellogg, whose resting eggs (or cysts) are abundant in the faeces and pellets of shorebirds at invaded salt pans in the Iberian Peninsula (Green et al., 2005).

Following their importance for use as nursery food in aquaculture, *A. franciscana* is spreading quickly around the Mediterranean region and other parts of the world, and displacing native brine shrimps including the sexually reproducing *Artemia salina* and a group of clonal *Artemia*, often grouped under the name *A. parthenogenetica* Bowen & Sterling (Amat et al., 2005; Van Stappen et al., 2007; Muñoz

& Pacios, 2010). This displacement is thought to have had a negative impact on natural cestode parasites of *Artemia*, which appear unable to switch to the exotic host (Georgiev et al., 2007). It may also reduce the foraging intake of birds using hypersaline wetlands, since they feed selectively on *Artemia* infected with cestodes (Sanchez et al., 2009).

Different hypotheses have been proposed to explain the capacity of invasive invertebrates to colonise quickly and outcompete native species. Among other factors, invasive species may flourish by escaping their natural enemies and parasites (Wolfe, 2002) and have a high population growth rate, short generation time and high dispersal ability (Williamson, 1996; Whitney & Gabler, 2008). The rapid spread of invasive organisms does indicate a high dispersal capacity (Williamson, 1996). In this study, to account for the rapid spread of *A. franciscana*, we present experiments to compare its dispersal potential via migratory shorebirds with that of the native *A. parthenogenetica*. We also compared the ability of the cysts of these two taxa to survive passage through the gut of migratory shorebirds, as well as their retention time, and their ability to become attached to feathers as shorebirds move around hypersaline habitats in which cysts are floating. We test the hypotheses that cysts of the invader are: (i) more likely to survive gut passage, (ii) more likely to hatch after retrieval from faeces, (iii) have a longer retention time within the gut, thus facilitating long-distance dispersal and (iv) have a greater propensity to become attached to feathers of shorebirds. We also compared the potential of two different shorebirds, varying in body size and migratory behaviour, as vectors for endozoochory: the Redshank *Tringa totanus* Linnaeus and Dunlin *Calidris alpina* Linnaeus. We predicted that cyst retention time would be longer in Redshank, owing to its larger size (Hahn, Bauer & Klaassen, 2008).

Methods

Fresh, intact cysts were collected in August 2005 at sites in Spain where native and invasive *Artemia* are under long-term study. *Artemia parthenogenetica* was collected from the Odiel salt ponds near Huelva (Sanchez, Green & Castellanos, 2006a for detailed description). *Artemia franciscana* were collected from La Tapa salt ponds near Puerto de Santa María in Cadiz Bay. Species identification has repeatedly been confirmed from these study areas by morphological and genetic criteria (Amat et al., 2005; Muñoz et al., 2010). The *A. parthenogenetica* at Odiel are predominantly diploid with a small percentage of tetraploid individuals, but we had no way of separating these in our sample.

In the laboratory, cysts were washed using distilled water (0.1-mm mesh sieve), dried for 24 h at 40 °C and then stored in the dark at 5 °C. To test the capacity of the two *Artemia* species to survive passage through the gut of migratory shorebirds, four Redshank (*T. totanus*) and six Dunlin (*C. alpina*) were captured in mist nets at El Pilar salt pans (Cádiz) in September–October 2005. These species, like Black-tailed Godwit (*Limosa limosa* Linnaeus, see below), are known to be habitual consumers of *Artemia* cysts in the wild (Green et al., 2005; Sanchez, Green & Castellanos, 2005). Experiments were carried out in Jerez Zoo, Spain in two phases on 8 September [four *T. totanus* and one *C. alpina*] and 6 October 2005 (five *C. alpina*). Licences and permits to catch the birds and to conduct the experiments were provided by Dirección General de Gestión del Medio Natural, Consejería de Medio Ambiente, Junta de Andalucía. The experimental design and procedures aimed to minimise the stress to the captive birds. Birds were previously acclimated to captivity in cylindrical cages (1.5 m diameter, 1 m high) divided into four compartments (one per bird) and covered by a 5-mm mesh net, allowing birds to maintain visual contact. Birds were kept outdoors under natural light for 1 week and provided with food (*Nereis polychaetes*) ad libitum.

For the feeding experiment, birds were placed indoors in individual boxes of 80 × 55 × 53 cm, one side being made from a 1-cm mesh net. Tissue paper was placed in the bottom of the cages to collect fresh faeces. Each bird was force-fed 1000 cysts using a syringe and administering a small amount of water (0.5 mL) to avoid regurgitation. Cysts remaining in the syringe were counted and discarded. Faeces were then collected at regular intervals (every 30 min for 0–2 h, every hour from 2 to 6 h, every 2 h from 6 to 14 h and then every 4 h until 24 h had passed since ingestion). Each individual bird was used only once and was fed either *A. parthenogenetica* or *A. franciscana* cysts. Half of the individuals of each bird species were fed with each of the brine shrimp species. Once the experiment was finished, birds were kept in captivity for 1 week to ensure that no cysts of *A. franciscana* remained in the digestive tract and were then liberated at the original place of capture.

On the day of collection, faeces were washed in the laboratory on a 0.1-mm sieve and resuspended in filtered hypersaline brine, which caused cysts to float. Intact cysts were retrieved and counted. The tissue paper used to collect faeces was also inspected for the presence of additional cysts. Immediately following cyst recovery, the hatchability of retrieved cysts was determined by placing them in a Petri dish (no more than 30 cysts per dish) filled with filtered diluted brine (28 g L⁻¹) and

incubated in a climate chamber at 25 °C under continuous light. The hatchability of control cysts from the same source was also assessed for comparison, using 20 replicates/Petri dishes per *Artemia* species, 10 containing 20 cysts and 10 containing 40 cysts each. Hatching was checked every day for the next 3 days. We considered that a cyst had hatched when it reached the ‘umbrella’ stage as the nauplius larva was emerging (Lavens & Sorgeloos, 1996). It is likely that many of the cysts that failed to hatch were viable, and lack of aeration meant that hatching success was probably suboptimal (Lavens & Sorgeloos, 1996).

Statistical analyses were performed using the GLIMMIX procedure (SAS Institute Inc., 2008) with a binomial error distribution and a logit link function. Survival of gut passage (defined as retrieval in an apparently intact state) by cysts (0 or 1) was analysed as the dependent binomial variable and *Artemia* and bird species as independent fixed factors. Hatching success of cysts (0 or 1) was analysed as the dependent binomial variable in separate analyses with *Artemia*, bird species, retention time and treatment (ingested by birds or control) as independent factors. In all analyses, statistical values were adjusted for model dispersion, while ‘individual bird’ was included as a random factor to control for pseudoreplication of cyst data coming from the same specimen. Retention time was compared between *Artemia* species and bird species using a gamma error distribution and logarithmic link function and fitting each individual bird as a random factor.

In a separate experiment, we used four Black-tailed Godwits found dead in Andalusia and frozen for later preparation as museum specimens. Breast feathers were removed after defrosting. Brine (100 g L⁻¹) collected from an Odiel salt pond where *Artemia* and shorebirds are

abundant was placed in a Petri dish, and then 50 cysts were floated on the brine. Holding a feather by the quill, it was then moved around amongst the cysts for 5 s with the convex side (which faces away from the bird under natural conditions) in contact with the water surface. The feather was removed and was then shaken five times by hand before inspection under a binocular microscope to count the number of cysts attached. The number of cysts remaining in the Petri dish were also counted, to establish how many cysts became attached initially but were lost during shaking.

This protocol was repeated 20 times each for cysts of the two *Artemia* species (source as above), using a different feather and different cysts each time. For each species, five different feathers were used from each of the four godwits. Differences between species were compared using a Mann–Whitney U-test carried out using Statistica 6.0 (StatSoft Inc., 2001).

Results

Artemia cysts were recovered from the faeces of all shorebirds apart from one *C. alpina* (which had ingested *A. franciscana*). Some of the cysts retrieved hatched from the other five *C. alpina*, as well as from one *T. totanus* which had ingested *A. franciscana*. In total, 4074 cysts of *A. parthenogenetica* were ingested, of which 8.4% were retrieved (Table 1). Similarly, a total of 3714 cysts of *A. franciscana* were ingested, of which 8.1% were retrieved. No statistically significant differences were found between *Artemia* species ($F_{1,7} = 0.01$, $P = 0.91$) or bird species ($F_{1,7} = 1.38$, $P = 0.28$) in the proportion of ingested cysts that were retrieved, although there was greater retrieval from *C. alpina* (Table 1).

Table 1 Mean ($\pm$ SE), mode, median and maximum retention time (in h) for retrieved (R) and hatched (H) cysts, and percentages retrieved and hatched for bird-ingested (I) and control (C) cysts. The experiment ended at 24 h. Hatchability is the percentage of intact cysts that hatched within 3 days

	Retention time								Retrieval (%)	Hatchability (%)
	Mean		Mode		Median		Maximum			
	R	H	R	H	R	H	R	H		
Tringa totanus Ap	1.4 ± 0.1	–	1.5	–	1.5	–	1.5	–	0.7 ± 0.4	0.0 ± 0.0
T. totanus Af	1.9 ± 0.2	1.5 ± 0.0	1.5	1.5	1.5	1.5	5.0	1.5	4.7 ± 1.6	2.0 ± 2.0
Calidris alpina Ap	1.2 ± 0.1	0.8 ± 0.1	0.5	0.5	0.5	0.5	10.0	6.0	13.5 ± 1.2	17.9 ± 5.9
C. alpina Af	1.2 ± 0.1	1.3 ± 0.1	1.5	1.5	1.0	1.5	12.0	2.0	10.4 ± 7.5	25.3 ± 6.5
Total T. totanus	1.9 ± 0.1	1.5 ± 0.0	1.5	1.5	1.5	1.5	5.0	1.5	2.7 ± 1.3	1.0 ± 1.0
Total C. alpina	1.2 ± 0.1	1.1 ± 0.1	0.5	0.5	0.5	0.5	12.0	6.0	11.9 ± 3.5	20.9 ± 4.3
Total Ap	1.2 ± 0.1	0.8 ± 0.1	0.5	0.5	0.5	0.5	10.0	6.0	8.4 ± 3.2	10.8 ± 5.5
Total Af	1.4 ± 0.1	1.3 ± 0.1	1.5	1.5	1.5	1.5	12.0	2.0	8.1 ± 4.4	13.7 ± 7.3
Control Ap	–	–	–	–	–	–	–	–	–	19.3 ± 3.6
Control Af	–	–	–	–	–	–	–	–	–	31.4 ± 4.0

Retrieved cysts (11–14%) had a lower hatchability than controls (19–31%, Table 1), but not significantly so ($F_{1,45} = 2.67$, $P = 0.11$). Similarly, there was no significant difference in hatchability between *Artemia* species ($F_{1,45} = 0.78$, $P = 0.38$), and no interaction between *Artemia* species and treatment (control or ingested, $F_{1,45} = 1.06$, $P = 0.31$). However, when analysing only cysts ingested by birds, cysts retrieved from *C. alpina* had a higher hatchability than those from *T. totanus* ($F_{1,6} = 7.84$, $P = 0.03$).

Cysts were retained in the gut for up to 12 h (Table 1). The mean retention time of retrieved cysts from a given bird was not related either to *Artemia* species ($F_{1,6} = 0.66$, $P = 0.45$; Fig. 1) or to bird species ($F_{1,6} = 0.95$, $P = 0.37$; Fig. 2). Of the cysts recovered, 84.3% of *A. parthenogenetica* and 89.6% of *A. franciscana* cysts were retrieved within 2 h of ingestion (Fig. 1). Hatchability of retrieved cysts did not vary significantly with retention time ($F_{1,585} = 0.05$, $P = 0.82$). This result did not change when controlling for

Fig. 1 Retention time for intact cysts of (a) *Artemia parthenogenetica* and (b) *Artemia franciscana*. Data represent mean values $\pm$ SE for different individual birds. White circles represent time intervals when no cysts were recovered.

Fig. 2 Retention time of intact *Artemia* cysts for (a) *Tringa totanus* and (b) *Calidris alpina*. Data represent mean values $\pm$ SE for different individual birds. White circles represent time intervals when no cysts were recovered.

Artemia and/or bird species ($F_{1,585} \leq 0.10$, $P \geq 0.75$), nor when fitting retention time as a quadratic function to allow for curvilinear relationships ($F_{1,584} \leq 2.48$, $P \geq 0.12$).

From the 40 trials involving 2000 cysts, initial attachment to feathers was recorded on five feathers involving a total of five cysts of *A. parthenogenetica* (on three feathers) and three of *A. franciscana*. After shaking, a total of one cyst of *A. parthenogenetica* and three of *A. franciscana* (on two feathers) remained attached. Differences between *Artemia* species were not significant (Mann–Whitney U-test, $P > 0.5$).

Discussion

There is an increasing interest in the role of birds as vectors of other organisms (Sekercioglu, 2006). To the best of our knowledge, this is the first study to compare the

retention time, survival and viability of the propagules of two invertebrates at the same time. Various studies have carried this out with seeds (Espinari et al., 2004; Brochet et al., 2010c; Figuerola et al., 2010). Malone (1965) compared the viability of eggs of two gastropod species after passing through the gut of ducks, but did not study retention time.

There was a great deal of variation between individual birds in retention time, the proportion of cysts that passed through the gut, and the proportion that hatched. In field conditions, the proportion of cysts destroyed decreases when shorebirds ingest fewer bivalves and other hard food items, and when they ingest a larger number of cysts (Sanchez et al., 2007). High variability between individual birds is typical in experiments in which seeds are retrieved from waterbird faeces (Brochet et al., 2010c; Figuerola et al., 2010) and led us to find few statistically significant differences, even when mean values were very different between treatments (Table 1). Similarly, no significant differences were observed between duck species in retrieval over time of propagules of the bryozoan *Cristatella mucedo* Cuvier or of the cladoceran *Bythotrephes longimanus* Leydig (Charalambidou, Ketelaars & Santamaría, 2003a; Charalambidou, Santamaría & Figuerola, 2003b), although these studies only used three or four individuals of each bird species.

Although invertebrate propagules can be transported externally on waterbirds (Brochet et al., 2010a), this study suggests that internal transport is more important for *Artemia* cysts, as also suggested for seeds transported by ducks (Brochet et al., 2010b). Cysts are smooth spheres and, although they float at the surface after release in salt ponds where waterbirds are swimming or feeding, our experiment suggests they have little propensity to stick to feathers. On the other hand, cysts form concentrations on the shoreline downwind, and birds may carry cysts on their feet with the help of sticky mud (Figuerola & Green, 2002b).

Both *Artemia* taxa are clearly capable of surviving passage through the gut, as previously indicated by the abundance of viable cysts in faeces collected from the field (Green et al., 2005; Sanchez et al., 2007). We found no evidence of differences in survival, retention time or hatchability between native and exotic *Artemia*. Hence, they may have a similar potential for dispersal by waterbirds that ingest cysts directly, or by preying on females with cysts in their ovisacs. Field observations suggest that shorebirds mainly ingest cysts by the latter mechanism (Sanchez et al., 2007). Our results suggest that the invasiveness of *A. franciscana* in the Mediterranean region is aided by a strong potential to disperse by

waterbirds, but that this is no greater than in the native *Artemia*. Other studies suggest that the invader can outcompete native *Artemia* by a combination of greater fecundity and release from natural parasites (Amat et al., 2007; Georgiev et al., 2007). However, our study cannot be considered a general test of the difference in dispersal potential between native and exotic invertebrates.

Artemia franciscana is also spread on cars or boots of people moving between salt pans (Waterkeyn et al., 2010). Genetic studies suggest that diploid *A. parthenogenetica* originated within the last 12 000 years in central Asia and spread quickly across the Mediterranean region, with birds being the most likely vector (Muñoz et al., 2010). They were able to exploit the numerous coastal salt pond complexes created around the Mediterranean for salt extraction since Roman times.

Cysts retrieved from *C. alpina* had a higher hatchability than those retrieved from *T. totanus*, possibly because their smaller gizzard caused less physical damage (Figuerola, Green & Santamaría, 2002). However, in the field, both species can regurgitate some food items as pellets. Field data suggest that *Artemia* cysts from *T. totanus* pellets have a higher hatchability than cysts found in faeces (Green et al., 2005; Sanchez et al., 2007). We did not observe any pellets during or after our experiment, but it remains possible that some pellets were produced after the birds were released, and that therefore we underestimated both survival of cysts and retention time in *T. totanus*. Proctor (1968) found that some shorebirds regurgitate seeds more than a week after ingestion.

Although we did not record retention times of more than 12 h, viable cysts of *A. franciscana* were retained for up to 24 h in the gut of Killdeer *Charadrius vociferus* Linnaeus (Proctor, Malone & deVlaming, 1967). Similarly, intact *Artemia* cysts were retained in the greater flamingo *Phoenicopterus ruber* Linnaeus and shelduck *Tadorna tadorna* Linnaeus for up to 38 h, and these two species are also likely to be major vectors of *Artemia* (MacDonald, 1980). The flamingo has recently become particularly abundant in Mediterranean sites frequented by *Artemia* (Rendón et al., 2008). The mean and maximum retention times of invertebrate eggs we recorded were relatively low compared to experiments with dabbling ducks *Anas* spp., which are larger birds. Mean retention time of *A. franciscana* cysts was 4 h in mallards *Anas platyrhynchos* Linnaeus, and the maximum was 28 h (Charalambidou et al., 2005), although this duck species is not known to be a vector of brine shrimps. Maximum retention time was 44 h for *C. mucedo* statoblasts (Charalambidou et al., 2003b) and 22 h for *B. longimanus* eggs (Charalambidou et al., 2003a).

Redshank and dunlin fly at 48–56 km h⁻¹, and therefore, our results indicate that most *Artemia* cysts could be dispersed less than 100 km, even if a bird takes flight shortly after feeding. However, this is enough to allow the spread of *A. franciscana* around the Mediterranean on a step-by-step basis between neighbouring salt pan complexes (Amat et al., 2005). Furthermore, the maximum retention time we observed suggests that cysts are sometimes dispersed up to 600 km. The most important barrier to the spread of the sexual *A. franciscana* is likely to be the Allee effect (Sarnelle & Knapp, 2004), especially when introduced into enormous populations of native brine shrimps.

The ecology of different waterbird species has a fundamental influence on their role as dispersal vectors (Green, Figuerola & Sanchez, 2002). Redshank and dunlin both have populations of hundreds of thousands of individuals moving through the Mediterranean region, but differ in their breeding range and migratory behaviour (Wetlands International, 2006) and are likely to have different roles in the dispersal of *Artemia*. Dunlin breed further north and only pass through the Mediterranean during migration, when they are best suited to disperse cysts between sites differing in latitude. Redshank are present in the region throughout the year, disperse cysts at various times of the year (Sanchez et al., 2007) and are well suited to moving shrimps between sites at differing longitude. However, ringing recoveries from the Odiel marshes show that many Redshank also move between this site and northern Europe (Sanchez, Green & Castellanos, 2006b).

There is no reliable information on the number of exotic invertebrate taxa introduced into different continents, largely because many are small, cryptic and only studied in detail in a few places. The dispersal of many of these species has been linked to transport by humans (Havel & Shurin, 2004; Holeck et al., 2004). In the case of invasive bivalves, such as the zebra mussel *Dreissena polymorpha* Pallas, experiments suggest they do not survive passage through the avian gut (Thompson & Sparks, 1977; Johnson & Carlton, 1996), although they could potentially be carried on the outside of birds (Green & Figuerola, 2005). However, a broad range of aquatic invertebrates are known to disperse via waterbirds, including rotifers, copepods, branchiopods, ostracods, bryozoans, nematodes and dipterans (Green & Sanchez, 2006; Frisch, Green & Figuerola, 2007; Brochet et al., 2010b), although there remains little information at the species level. Hence, we suggest that birds have a role in the spread of numerous exotic species, and *A. franciscana* gives us an example of how readily waterbirds can act as vectors.

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