

Research Article

Influence of developmental stage and sex on infection of the American brine shrimp *Artemia franciscana* Kellogg, 1906 by avian cestodes in Ebro Delta salterns, Spain

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Abstract

The American brine shrimp *Artemia franciscana* Kellogg, 1906 is invasive in the Mediterranean region where it has displaced native populations of sexual *A. salina* and clonal *A. parthenogenetica* from many hypersaline wetlands. Brine shrimps are intermediate hosts of several cyclophyllidean avian cestodes, whose effects have been studied in native *Artemia*. However, determinants of these infections in the alien invader remain unknown. We present the most detailed study to date of parasitism of *A. franciscana* by cestodes in its invasive range, conducted in the Ebro Delta salterns (NE Spain) over a 33 month period, when a total of 9,293 *A. franciscana* adults and 8,902 juveniles were studied. We examined for first time whether host age and sex influence infection rates. Ten cestode species were recorded, with a total prevalence of 11.1% in adults and 2.7% in juveniles. The most abundant cestodes were *Eurycestus avoceti* (in adults) and *Flamingolepis flamingo* (in juveniles). Prevalence, abundance and intensity of infection were significantly higher in adults for four cestode species (*Flamingolepis liguloides*, *Wardium stelleriae*, *E. avoceti* and *Anomotaenia microphallos*), and significantly higher in juveniles for *F. flamingo*. Mean cestode species richness was four times higher in adults. One cestode species (*F. liguloides*), was significantly more abundant in adult females than in adult males. Host age influences infection patterns which seem to be strongly related to the feeding behaviour of avian final hosts (filtering versus picking), suggesting complex ecological interactions among predators, prey and their parasites, with important implications for native biodiversity and ecosystem stability.

Key words: host-parasite interactions, helminth larvae, intrinsic factors, age, invasion process

Introduction

Brine shrimps of the genus *Artemia* Leach, 1819 (Crustacea, Branchiopoda, Anostraca) are a complex of sibling species distributed worldwide with the exception of Antarctica (Vanhaecke et al. 1987; Triantaphyllidis et al. 1998). The American brine shrimp *Artemia franciscana* Kellogg, 1906 is a key species for the aquaculture industry, serving as live food for fish larvae and crustaceans (Van Stappen 1996). *A. franciscana* cysts, particularly originating from Great Salt Lake and San Francisco Bay (USA), have been exported for aquaculture, the improvement of salt extraction in salterns, the pet trade market, and to generate local sources

of cysts (dormant eggs) (Abatzopoulos et al. 2002; Sui et al. 2012), facilitating the arrival and spread of *A. franciscana* outside its natural range (Amat et al. 2005). *A. franciscana* is highly invasive and usually rapidly outcompetes native *Artemia* species possibly due to its higher reproductive rate and competitive abilities, and its markedly eurythermal and euryhaline character (Browne and Halanych 1989; Browne and Wanigasekera 2000; Amat et al. 2007). It also has a high capacity to disperse between habitats via migratory waterbirds, which might facilitate its expansion (Sánchez et al. 2012a; Muñoz et al. 2013). In the Mediterranean, the introduction of this exotic species is provoking the extinction of native *A. salina* (Linnaeus, 1758)

and *A. parthenogenetica* Bowen and Sterling, 1978 populations (Amat et al. 2007; Ben Naceur et al. 2010; Pinto et al. 2013; Muñoz et al. 2014). *A. franciscana* has also spread into Asia (Iran, Irak, India, Paquistán, China, Vietnam), and Australasia (Ruebhart et al. 2008; Vikas et al. 2012), being a threat for native biodiversity (mostly parthenogenetic forms and bisexual species such as *A. urmiana* Günther, 1899 in Ukraine and Iran, or *A. sinica* Cai, 1989 in China).

Artemia spp. are intermediate hosts of avian cestodes that can have a major influence on their fitness, with a total of 15 species recorded in the Palaearctic *Artemia* species (Redón et al. 2015a). In the Western Mediterranean, native sexual *A. salina* and asexual *A. parthenogenetica* are parasitized by 12 species of tapeworms whose final hosts are flamingos, waders, grebes, ducks or gulls (Georgiev et al. 2005; Vasileva et al. 2009; Sánchez et al. 2013a). Although *A. franciscana* was introduced as cysts, and therefore free of its own native helminth parasites from North America, it may be infected by parasites using other *Artemia* spp. as intermediate host in Mediterranean wetlands. So far, nine cestode species have been found in introduced *A. franciscana* populations (Georgiev et al. 2007; Georgiev et al. 2014; Sánchez et al. 2012b; Redón et al. 2015a). A recent study in Great Salt Lake (Utah, USA) has recorded the presence of four cestode and one nematode species parasitizing *A. franciscana* in its native range (Redón et al. 2015b). The role of cestodes in the ecology and biology of their brine shrimp hosts has been addressed mainly by studies of native *Artemia* (Sánchez et al. 2013b). Almost all previous studies focus on adult brine shrimps, and there is little knowledge of the role of earlier developmental stages of *Artemia* in the life cycle of avian cestodes (Redón et al. 2011). There are relatively few studies of cestodes affecting *A. franciscana* in its invasive Mediterranean range, and here we present the most detailed study to date.

In the present work, our first objective was to study the cestode community in the alien invasive *A. franciscana* inhabiting the Ebro Delta salterns in order to understand the role of this invader as an intermediate host to native parasites (Sánchez et al. 2012b; Georgiev et al. 2007, 2014; Redón et al. 2015a). Secondly, we aimed to explore if host age class (in terms of developmental stage: juvenile or adult) and host sex influence parasite infection rates. To do that, we assessed cestode infections for juvenile and adult *Artemia* separately in order to provide the first study of the potential role of *A. franciscana* juveniles as intermediate

hosts in the circulation of avian cestodes. We tested the hypothesis that there is an accumulative effect of parasites with the development of the intermediate host, such that adults have consistently higher infection rates than earlier stages. We compared the infections in adult males and females to test the hypothesis that females would be more prone to infections owing to their longer lifespan (based on observations from life table studies: S. Redón and F. Amat, unpubl. data) and larger size (Amat 1985; Amarouayache et al. 2009a) compared to males; this might result in greater exposure to parasites and provide more resources to incoming parasites (Poulin 1996). Finally, we consider how the cestode community and their temporal dynamics related to the community of avian final hosts in the study area.

Materials and methods

Study system and field samples

The Ebro Delta (Province of Tarragona, NE Spain) is the largest wetland area (320 km²) on the Mediterranean coast of Spain and is protected as a Natural Park, Ramsar site and an EU Special Area for Bird Protection. It is one of the most important habitats for migratory waterbirds in the Iberian Peninsula (Martí and Del Moral 2002). The alien species *Artemia franciscana* was first detected in our study area (*La Trinitat* coastal saltern complex, 40°35'N, 00°41'E, Figure 1) in 2007 and its introduction probably originated as a result of prawn larviculture activities in the area (Amat et al. 2007). Previously these salterns supported a tetraploid parthenogenetic population of *Artemia* (Amat et al. 1995), but this native form has not been recorded since.

Repeated sampling visits were carried out from 2007 to 2010. Live *Artemia* were collected in five ponds (Figure 1) chosen according to accessibility and *Artemia* availability. Surface temperature (T) and salinity (S) were measured *in situ* with a hand-held thermometer and Shibuya® refractometer (measurements taken from 11:00 h – 13:00 h). *A. franciscana* populations were found in all visits, although they were usually near the bottom of the water column and difficult to detect visually.

Sampling efforts were concentrated in the following three ponds (depth 20 – 50 cm) with the aim of studying the temporal dynamics of *Artemia* and their parasites (Figure 1). Pond 4 was sampled monthly from June 2007 to March 2009, then in January and March 2010 (S range = 85 – 170 ‰, mean ± s.e. = 114.8 ± 4.57). Pond 6 was sampled monthly from March 2008 to March 2009, except



Figure 1. Geographic location of the Ebro Delta (Province of Tarragona, NE Spain) and map of the Ebro Delta salterns *La Trinitat* indicating the *Artemia* collection sites: (A) Pond 4, (B) Pond 6, (C) Pond CX, (D) Evaporator 3, (E) Channel.

in November (S range 160–280‰, mean = 214.1 ± 14.03). Pond CX, a large pond between the other salt ponds and the sea, isolated from the brine circulation system (Figure 1), was the only pond in which *A. franciscana* regularly coexisted with the native *A. salina* (details of cestode infections in these native shrimps are presented by Redón et al. 2015a). Samples were collected monthly from January 2009 to January 2010 (S range = 45–260‰, mean \pm s.e. = 114.6 ± 18.35). Brine T ranged between 5 and 32°C (mean \pm s.e. = 18.5 ± 2.67 , considering these three ponds). Additional samples were also collected in April (T=23°C, S=264‰) and October 2008 (T=14°C, S=240‰) from a canal between evaporation and crystallization ponds, and in March 2008 from Evaporator 3 (Figure 1, Figure S1).

Two *Artemia* samples were collected from each pond with 160 μ m and 500 μ m mesh hand-nets (sample A and sample B, respectively). Sample A was used to examine population age structure, whereas sample B was collected to ensure enough individuals for parasitological analyses. Animals were transferred into several 20 L plastic containers filled with pond brine while some fresh cultured microalgae (*Dunaliella* sp. + *Tetraselmis suecica*) were added. Then, samples were transported to the laboratory where they were maintained for several days in 60 L tanks with 100 % brine and

gentle aeration, and fed on the same mixed microalgae culture.

Brine shrimp host populations: species and age structure

Within 48h, a subsample of 2 L from sample A was sieved through a 200 μ m mesh, then *Artemia* were lightly anaesthetized in seawater with some drops of chloroform-saturated distilled water, examined under a stereomicroscope and assigned to four age-classes (nauplii, metanauplii, juveniles and adults). Juveniles are immature specimens with sexual segments (ovisac or hemipenis) that are not completely developed but enough to be identified at species-level.

Juveniles and adults were identified to species following morphological descriptions (Amat 1985). The proportion of juveniles varied over time (Figure S1) confirming that this alien species had multiple generations per year (Amat et al. 2007). We separated the sexes in juveniles and adults (Amat 1985).

Parasite identification

Juveniles and adults from sample A were examined while anaesthetized under a stereomicroscope for cestode cysticercoids. Further individuals were

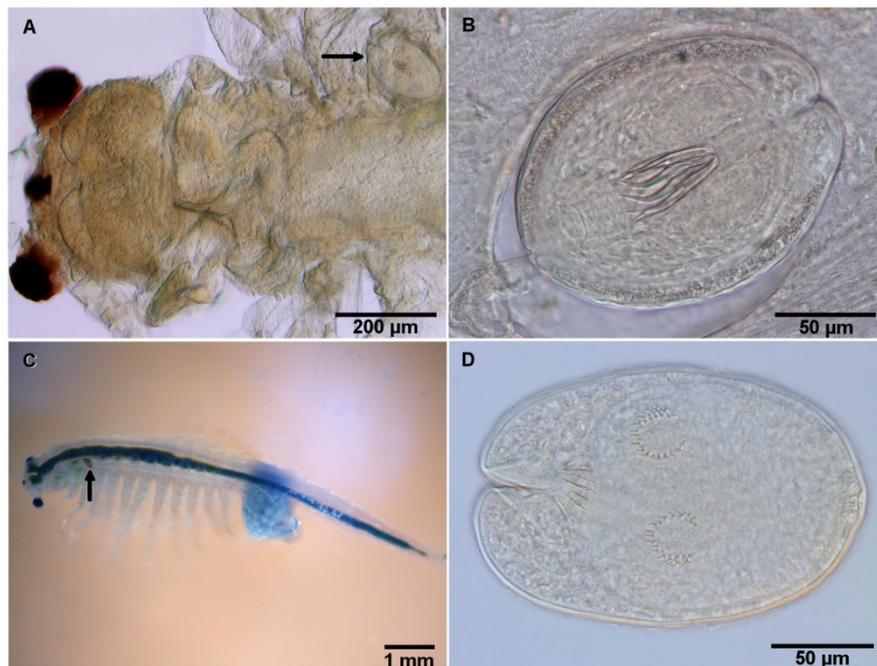


Figure 2. General aspect of the brine shrimp *Artemia franciscana* infected by the most prevalent parasite species in each developmental class. A) Juvenile male infected by *Flamingolepis flamingo* (arrow) and (B) detail of the cysticercoide. C) Adult female infected by *Eurycestus avoceti* (arrow) and (D) detail of the cysticercoide.

randomly selected from sample B until a combined total of approximately 500 individuals (when available) was reached, including juveniles and adults. After observations of the cysticercoide *in situ*, each infected specimen was prepared in a temporary glycerol mount and examined under a compound microscope. When a more accurate examination was needed, isolated cysticercoide were mounted in permanent Berlese's medium slides to facilitate observations of the rostellar hooks. Identification was based on Georgiev et al. (2005) and Vasileva et al. (2009).

Quantitative analysis of cestode infection and statistics

Several descriptors were considered in order to quantify cestode infections in *Artemia*. Prevalence (P%: proportion of infected individuals in the host population), abundance (MA: mean number of cysticercoide for the total number of shrimps examined) and intensity (MI: mean number of cysticercoide in infected shrimps) were calculated for the overall infection and for each parasite species (terminology following Bush et al. 1997). Also, we quantified species richness (SR: mean number of cestode species present in each *Artemia* individual) and the species richness in infected specimens (SR infected), as well as the relative abundance (RA) of each cestode species (as a proportion of all cysticercoide).

Host age: adults vs. juveniles

The age of *Artemia* individuals was assessed by their developmental stage which is also related to size ranges: 4–6 mm for juveniles and > 6 mm for adults (larval stage X–XII and XII–XVIII, respectively; see Amat 1985) rather than by days since hatching because in the natural populations we had no way of knowing dates of hatching. We analyzed parasites in *A. franciscana* adults and juveniles separately, and for 46 samples in which both age groups occurred. We compared infection levels using Wilcoxon Paired tests, for prevalence, MI, MA and SR.

Sexual differences in parasite loads

Differences in infection parameters between male and female adult *Artemia* present in a given sample were tested with Wilcoxon Signed Rank tests.

Results

Cestode community in Artemia franciscana and seasonal variation

We examined a total of 18,195 specimens of *A. franciscana* (9,293 adults and 8,902 juveniles) sampled from June 2007 to March 2010, and 1,277 of which were infected with cestode cysticercoide (total prevalence 7%). The size of juveniles ranged

from 3.6 to 6.8 mm, while for adults the mean size \pm s.d. was: 7.8 ± 0.65 mm for ovulating females, 8.68 ± 0.66 mm for ovigerous females, and 7.59 ± 0.42 mm for males ($N = 15$ in each group). We recorded a total of 1,618 cysticercoids of 10 cestode species: *Flamingolepis liguloides* (Gervais, 1847), *Flamingolepis flamingo* (Skrjabin, 1914) (Figure 2 A, B), *Gynandrotaenia stammeri* Fuhrmann, 1936 and *Gynandrotaenia* sp. (adults parasitic in flamingos); *Wardium stellorae* (Deblock, Biguet et Capron, 1960) and *Branchiopodataenia gvozdevi* (Maksimova, 1988) (gulls); *Fimbriarioides tadornae* Maksimova, 1976 (shelducks); *Anomotaenia tringae* (Burt, 1940), *Anomotaenia microphallos* (Krabbe, 1869) and *Eurycestus avoceti* Clark, 1954 (Figure 2 C, D) (waders). One species, *E. avoceti*, was dominant, with a relative abundance (RA) of 56.5% and peaks of infection in July-August, particularly in the case of adult *A. franciscana* (Figure 3). The temporal dynamics of cestode infection indicate higher prevalences in summer months for two consecutive years of the study period (2007 and 2008). The same seasonal pattern was observed for *E. avoceti*, the most abundant parasite in the community (Figure 3).

Comparing cestode infections in adult and juvenile *A. franciscana*

Although cestode infections were recorded in juveniles, adults were more likely to be infected. For all specimens examined, 11.1% of adults were infected, compared to 2.7% of juveniles (Table 1). The total cestode intensity ranged from 1–7 in adults and 1–4 in juveniles (although $< 2\%$ of infected individuals had more than 3 and 2 cysticercoids for adults and juveniles, respectively). Although all 10 cestode species occurred in both age groups, their relative abundance was markedly different (Table 1, Figure S2). The dilepidid *E. avoceti* dominated in adults (RA = 64.1%), and the hymenolepidid *F. flamingo* in juveniles (RA = 52.8%) (see also Figure 2).

For 46 samples in which both adults and juveniles were present, overall cestode infection, prevalence and total cestode abundance were significantly higher in adults, although there was no difference in overall intensity (Table 1). Prevalence, abundance and intensity were also significantly higher in adults for four cestode species: *F. liguloides*, *W. stellorae*, *E. avoceti* and *A. microphallos* (Table 1, Figure 4). Intensity was also significantly higher in adults for *G. stammeri*. In contrast, for *F. flamingo*, prevalence, intensity and abundance were significantly higher in juveniles (Table 1).

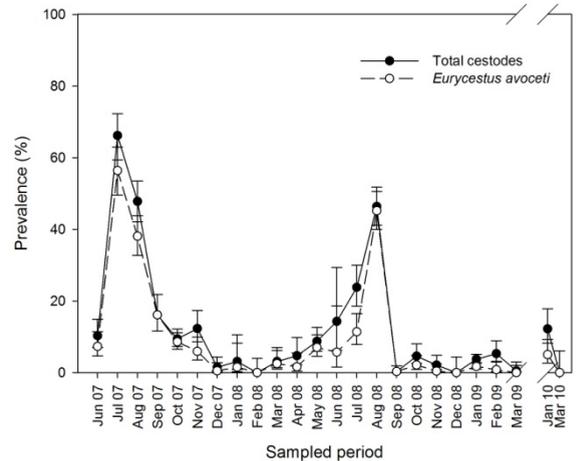


Figure 3. Seasonal prevalence of cestodes in *A. franciscana* adults from pond 4, from June 2007 to March 2010. Bars show 95% confidence intervals.

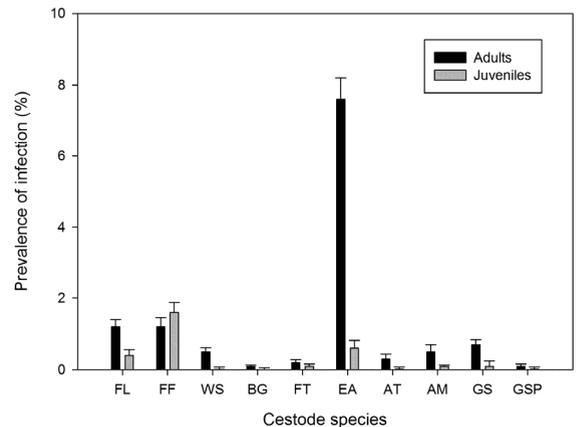


Figure 4. Prevalence of cestode species in alien *A. franciscana* from Ebro Delta according to age class (all available data, $N = 18,195$). Bars show upper 95% confidence intervals. FL, *Flamingolepis liguloides*; FF, *F. flamingo*; WS, *Wardium stellorae*; BG, *Branchiopodataenia gvozdevi*; FT, *Fimbriarioides tadornae*; EA, *Eurycestus avoceti*; AT, *Anomotaenia tringae*; AM, *A. microphallos*; GS, *Gynandrotaenia stammeri*; GSP, *Gynandrotaenia* sp.

The mean cestode species richness (SR) in adult *A. franciscana* was four times that observed for juveniles ($p = 0.002$), although there was no significant difference in SR of infected specimens (Table 1).

Cestode infections in male and female *A. franciscana*

In adult *A. franciscana* ($N = 5,473$ males, 3,820 females), prevalence of cestodes was lower in males (8.8%) than in females (14.5%), but no significant differences were found between sexes

Table 1. Cestode infections in adult and juvenile *Artemia franciscana*. FL, *Flamingolepis liguloides*; FF, *Flamingolepis flamingo*; WS, *Wardium stellorae*; BG, *Branchiopoddataenia gvozdevi*; FT, *Fimbriarioides tadornae*; EA, *Eurycestus avoceti*; AT, *Anomotaenia tringae*; AM, *Anomotaenia microphallos*; GS, *Gynandrotaenia stammeri*; GSP, *Gynandrotaenia* sp. N, total number of brine shrimp examined; P%, prevalence; MI, mean intensity; Max., maximum intensity; MA, mean abundance; RA%, relative abundance. \pm SE, standard error. Where there are significant differences in infection parameters between adults and juveniles according to Wilcoxon Signed Rank Tests, the higher values are shown as * $p < 0.05$, ** $p < 0.001$.

Cestode species	<i>Artemia franciscana</i> -adults N= 9,293					<i>Artemia franciscana</i> -juveniles N= 8,902				
	P%	Intensity		Abundance		P%	Intensity		Abundance	
		Max.	MI \pm SE	RA%	MA \pm SE		Max.	MI \pm SE	RA%	MA \pm SE
Hymenolepididae										
<i>Flamingolepis liguloides</i> -FL	1.2*	6	1.34 \pm 0.07*	10.9	0.016 \pm 0.002*	0.4	3	1.14 \pm 0.07	14.8	0.005 \pm 0.001
<i>Flamingolepis flamingo</i> -FF	1.2	3	1.04 \pm 0.02	8.8	0.013 \pm 0.001	1.6*	2	1.06 \pm 0.02*	52.8	0.017 \pm 0.001*
<i>Wardium stellorae</i> -WS	0.5*	2	1.05 \pm 0.03*	3.3	0.005 \pm 0.001*	0.02	1	1.00 \pm 0.00	0.7	0.0002 \pm 0.0002
<i>Branchiopoddataenia gvozdevi</i> -BG	0.1	1	1.00 \pm 0.00	0.4	0.001 \pm 0.0002	0.01	1	1.00	0.4	0.0001 \pm 0.0001
<i>Fimbriarioides tadornae</i> -FT	0.2	1	1.00 \pm 0.00	1.2	0.002 \pm 0.0005	0.1	1	1.00 \pm 0.00	2.5	0.001 \pm 0.0003
Dilepididae										
<i>Eurycestus avoceti</i> -EA	7.6**	6	1.20 \pm 0.02**	64.1	0.092 \pm 0.004**	0.6	2	1.05 \pm 0.04	20.8	0.007 \pm 0.001
<i>Anomotaenia tringae</i> -AT	0.3	3	1.07 \pm 0.07	2.2	0.003 \pm 0.001	0.02	2	1.50 \pm 0.50	1.1	0.0003 \pm 0.0003
<i>Anomotaenia microphallos</i> -AM	0.5*	2	1.02 \pm 0.02*	3.7	0.005 \pm 0.001*	0.1	1	1.00 \pm 0.00	1.8	0.001 \pm 0.0003
Progynotaeniidae										
<i>Gynandrotaenia stammeri</i> -GS	0.7	2	1.05 \pm 0.03*	4.8	0.007 \pm 0.001	0.1	1	1.00 \pm 0.00	4.6	0.0015 \pm 0.0004
<i>Gynandrotaenia</i> sp.-GSP	0.1	1	1.00 \pm 0.00	0.5	0.001 \pm 0.0003	0.02	1	1.00 \pm 0.00	0.7	0.0002 \pm 0.0002
Total cestode infection	11.1*	7	1.29 \pm 0.02		0.144 \pm 0.005*	2.7	4	1.16 \pm 0.03		0.032 \pm 0.002
Species Richness (SR) \pm SE			0.12 \pm 0.00*					0.03 \pm 0.00		
SR infected \pm SE			1.11 \pm 0.01					1.09 \pm 0.02		

for total prevalence, prevalence of any parasite species, or SR (Wilcoxon Signed Rank tests, comparing 49 paired samples, $p > 0.05$). In contrast, intensity of infection of *F. liguloides* was significantly higher in females (MI: 1.48 ± 0.15 , maximum = 6) than in males (MI: 1.24 ± 0.06 , maximum = 3, Wilcoxon Signed Rank test, 9 paired samples, $T = 1$, $p = 0.046$). Similarly, the abundance of *F. liguloides* was significantly higher in females (MA: 0.0188 ± 0.006) than in males (MA: 0.0097 ± 0.004 , mean \pm s.e. for 49 paired samples, $T = 88$, $p = 0.025$).

Discussion

Cestode community and temporal dynamics in A. franciscana

Our study of cestode infections in *A. franciscana* is the most complete to date in terms of its duration and the number of individuals and samples analyzed. We recorded 10 species of cestodes in *A. franciscana* in the Ebro Delta salterns compared to a previous local maximum in any locality of 6 species (at Castro Marim, Portugal; Georgiev et al. 2007). This is also the first record for *Wardium stellorae* (a cestode specific to gulls) in this alien brine shrimp. Grouping parasites according to their definitive hosts, 62% of all cysticercoids were parasites of shorebirds (*Eurycestus avoceti* + *Anomotaenia*

tringae + *A. microphallos*), 33.4% were parasites of flamingos (*Flamingolepis liguloides* + *F. flamingo* + *Gynandrotaenia stammeri* + *Gynandrotaenia* sp.), 3.2% were parasites of gulls (*Wardium stellorae* + *Branchiopoddataenia gvozdevi*), and 1.4% were a parasite of shelducks (*Fimbriarioides tadornae*). This is consistent with data from other *A. franciscana* populations in Spain, Portugal and France, in which parasites of shorebirds had the highest prevalence and abundance (Georgiev et al. 2007; Georgiev et al. 2014; Sánchez et al. 2012b). The species composition of the cestode community generally matches the structure of the waterbird community in the Ebro Delta (Martí and del Moral 2002; Supplementary Table S1).

As in other salterns in the Iberian Peninsula (Sánchez et al. 2013a), the greater flamingo *Phoenicopterus roseus* Pallas, 1811 was very abundant in our study area (Table S1) and dominant in terms of final host biomass. The greater abundance of shorebird parasites in *A. franciscana* suggests that this species has resistance to flamingo parasites. This and other studies show that *F. flamingo* is consistently more prevalent than *F. liguloides* in alien *A. franciscana* (Georgiev et al. 2007; Georgiev et al. 2014), unlike native *Artemia* in which *F. liguloides* is consistently more prevalent than any other cestode (Georgiev et al. 2007; Sánchez

et al. 2013a). Since both *Flamingolepis* species share the same final host, this suggests that *F. flamingo* is more pre-adapted to infect *A. franciscana*. Furthermore, *F. liguloides* has a stronger effect on the fecundity of native *A. salina* than on the invasive *A. franciscana* (Redón et al. 2015a).

Although care is required in interpreting data from only one sample per month collected in one part of the pond, we detected strong seasonal dynamics, with a strong peak in the prevalence of total cestode infections and infection by *E. avoceti* in July and August, which was repeated between years (Figure 3, S3). This is likely to be connected with dynamics of the final hosts, since shorebirds use the study area in large numbers during post-breeding migrations at this time of year (Figuerola and Bertolero 1996; Bigas 2012). Also, in *A. franciscana* juveniles, a peak in prevalence of total cestodes and prevalence of *F. flamingo* was observed in October (Figure S4), probably linked with higher abundance of flamingos in autumn and winter months (A. Curcó pers. comm.; unpublished data).

Brine shrimp age and sex effects on cestode infections

This study is the first exploring the role of juvenile stages of alien *A. franciscana* in the transmission of native cestodes in an invaded wetland. Juveniles were regularly infected by cestodes, though to a lesser extent than adults (Table 1). These differential infection rates between juvenile and adult shrimps suggest the accumulation of parasites with advancing host development. An age effect was previously detected in *A. parthenogenetica*, with total prevalence of 9.5%, 11.8%, and 50.3% for metanauplii, juveniles, and adults, respectively (Redón et al. 2011).

Our results have revealed a differential distribution of cestode species between age groups, suggesting that each parasite is adapted to infect hosts of a certain developmental stage, perhaps owing to differences in final host feeding behaviour (Redón et al. 2011). In particular, we found higher intensity and abundance of *F. flamingo* in juvenile *A. franciscana* than in adults. Reduced infections in adults suggest that infected juveniles are preferentially removed from the population by filter-feeding flamingos, although we cannot rule out the possibility that their development into adults is somehow delayed. In contrast, *E. avoceti* was more abundant in adult *A. franciscana*, and this cestode parasitizes shorebirds such as black-winged stilt (*Himantopus himantopus* Linnaeus, 1758) or pied avocet (*Recurvirostra avosetta* Linnaeus, 1758), which visually pick-out *Artemia* individually and

are more likely to feed on larger adult individuals, which are easier to detect and energetically more profitable. *A. microphallos* and *W. stellorae* were also more prevalent in adults, and these are also parasites of final hosts (gulls and shorebirds) that feed in such a manner.

However, our results may also be influenced by differences between cestode species in changes in the immune response of *Artemia* with age. For example, juvenile *A. franciscana* may be particularly susceptible to *F. flamingo* infection at an early stage. Selective age infection may also be determined by differences in parasite egg size (Redón et al. 2011). Differences in *Artemia* ability to filter particles across a size range is related with developmental stage (Gelabert et al. 2001), which suggests that parasites with bigger eggs are more likely to infect adult *Artemia*. However, in this case, eggs of both *Flamingolepis* species are small enough to be ingested by early stages of brine shrimp such as metanauplii and juveniles (Redón et al. 2011); thus, further studies are needed to clarify why *F. liguloides* infect juvenile *A. franciscana* to a relatively lower extent than its congener *F. flamingo*.

No sex-based differences in the overall infection rate were detected in *A. franciscana*. A slight sex difference was only identified in the case of *F. liguloides*, females being more intensely parasitized than males. This may be because the longer lifespan (unpublished data) and larger size of females increase the likelihood of ingestion of cestode eggs or provide a better habitat (in terms of space and nutritional resources) for development of the infective larval stage of this cestode species, which has a particularly large cysticeroid (see Georgiev et al. 2005). Another possible explanation is related to differences in immune defences between *Artemia* sexes. Sex-based differences in parasitism are extensively studied in vertebrates, and males tend to have significantly higher parasite prevalence and intensity than females (Poulin 1996; Schalk and Forbes 1997; Moore and Wilson 2002), probably due to the cost of sexual selection. Different exposure to parasites or an immune suppression associated with androgens (primarily testosterone) are thought to play a major role in this general male-bias pattern (Zuc and McKaen 1996). Among invertebrates, male-biases are also observed but owing to the absence of testosterone a different mechanism must be responsible for increasing male vulnerability (Zuc and McKaen 1996). For example, male copepods *Macrocylops albidus* (Jurine, 1820) infected experimentally by the larval cestode *Schistocephalus*

solidus (Müller, 1776) had significantly higher prevalence and intensity of infection than females, likely due to a weaker immune response in males (Wedekind and Jakobsen 1998). Sheridan et al. (2000), however, did not find a consistent sex bias in parasitic infections in arthropod hosts and suggested that the lack of steroid hormones could be a reason why males are not generally more parasitized than females. Differences in physiology, morphology, ecology and behaviour between males and females may all contribute to sex differences in parasite loads. The longer lifespan and larger size of *Artemia* females are likely to explain the higher intensity and abundance of *F. liguloides* in *A. franciscana* females.

Parasite implications in the invasion process

The invader *A. franciscana* population inhabiting the Ebro delta is characterized by a more diverse cestode assemblage than those observed in other areas of the introduced or native range. Indeed, in the Western Mediterranean it is infected by the same cestode species as native *Artemia* spp. but with a much lower overall rate of infection (Sánchez et al. 2013a; Redón et al. 2015a). In the Ebro Delta, the prevalence of *F. liguloides* and *A. tringae* is higher in *A. salina*, and both these species reduce the fecundity of their hosts (Amarouayache et al. 2009b; Redón et al. 2015a). In particular, the highest prevalence of *F. liguloides* is 60% for *A. salina* and 6.3% for *A. franciscana* (Redón et al. 2015a) suggesting that this parasite is a strong regulator of native host population dynamics. This suggests that resistance to cestodes could be explaining (at least partially) the invasiveness of *A. franciscana* in the Ebro delta salterns and other invaded ecosystems across the Old World. Further research on both cestode – *Artemia* interactions and effects in upstream bird hosts are needed to elucidate the role of parasites in the successful invasion of *A. franciscana*.

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The following supplementary material is available for this article:

Table S1. Annual total and mean counts of waterbird species at Ebro Delta Natural Park (NE, Spain) for the study period.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2015/Supplements/AI_2015_Redon_etal_Supplement.xls