

Larval helminths in the invasive American brine shrimp *Artemia franciscana* throughout its annual cycle

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Abstract

One of the best examples of rapid displacement of native species by an invader is the eradication of native *Artemia salina* and *A. parthenogenetica* in the Mediterranean by the introduced American *A. franciscana*. Previous studies based on sampling from limited time periods suggest that the success of the American species as a competitor may be due partly to different parasite burden, since native *Artemia* spp. have high cestode infection rates regulating their density. The aim of this study is to test the hypothesis that the helminth infection in *A. franciscana* in its invasive range is low throughout its annual life cycle. Samples of *A. franciscana* were collected every second month from La Tapa saltern (Andalusia) during one year. Five helminth species were recorded: cestodes *Flamingolepis liguloides*, *F. flamingo*, *Gynandrotænia stammeri* (all flamingo parasites), *Eurycestus avoceti* (a shorebird parasite) and larval spirurids of the Acuariinae (the first record of nematodes in *Artemia*). The overall infection rate was low, with total prevalence 5.9% and prevalence of individual parasite species between 0.2 and 3.2%. The mean abundance of helminths was 0.005–0.155 (av. 0.068), 5–13 times lower than in native congeners. Waterbird counts indicate that the low infection rates cannot be explained by lack of definitive hosts. The results are consistent with the hypothesis that helminths have no regulating effect on the invasive brine shrimp in the Mediterranean. The replacement of the native populations by the invader can be partially explained by a competition mediated by parasites/predators through a differential impact on host fitness.

Keywords

Artemia, cestodes, nematodes, biological invasion, hypersaline wetlands, salt ponds

Introduction

Comparative studies of parasite infections in native and alien host species are important to understand the role of parasites in biological invasions (Dunn 2009; Kelly *et al.* 2009; Dunn *et al.* 2012). Brine shrimps of the genus *Artemia* Leach, 1819 (Crustacea: Branchiopoda) from hypersaline Mediterranean wetlands and their helminth parasites offer a promising host-parasite system for elucidating effects of parasitism on the outcome of competitive interactions during an ongoing biological invasion. The American brine shrimp *A. franciscana* Kellogg, 1906, originating from commercialised populations at the San Francisco Bay and Great Salt Lake in North America (Muñoz *et al.* 2014), has been introduced into the Western Mediterranean and has expanded its range since the

1980s, displacing the native brine shrimps *A. parthenogenetica* Barigozzi, 1974 and *A. salina* (L., 1758) (Amat *et al.* 2005, 2007).

Native brine shrimps in the Mediterranean are intermediate hosts of 12 species of avian cestodes parasitic as adults in flamingos, gulls, grebes, shorebirds or ducks (Gabrion *et al.* 1982; Thiéry *et al.* 1990; Robert and Gabrion 1991; Amat *et al.* 1991b; Mura 1995; Georgiev *et al.* 2005, 2007; Vasileva *et al.* 2009; Amarouayache *et al.* 2009). Both adult shrimps and metanauplii participate in cestode life cycles (Redón *et al.* 2011). The presence of larval tapeworms (cysticercoids) in the native brine shrimps is associated with modifications of their colouration (Thiéry *et al.* 1990; Amat *et al.* 1991a; Sánchez *et al.* 2006a) and altered surfacing and photophobic behaviour (Sánchez *et al.* 2007), thus increasing the probability of being

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eaten by definitive hosts (Sánchez *et al.* 2009a). The infections with cysticercoids are linked with neurological and physiological disorders of intermediate hosts, changing the lipid content and respiration rate (Amat *et al.* 1991a; Varó *et al.* 2000; Sánchez *et al.* 2009b). Infected brine shrimps exhibit enhanced swarming propensity that may also increase the probability for transmission by predation (Rode *et al.* 2013). Isotopic studies indicate that cysticercoid infections can change the selective utilisation of trophic resources by native brine shrimps (Sánchez *et al.* 2013a). Furthermore, cestode larvae reduce the fecundity of the infected *Artemia* (Amat *et al.* 1991a; Sánchez *et al.* 2012).

Two previous studies in the Western Mediterranean have demonstrated that cestode infection rates in invasive brine shrimps are lower than in their native congeners. Georgiev *et al.* (2007) screened cestode infections in 7 populations of *Artemia* spp. (3 invasive and 4 native) along the southern Iberian coast in summer 2005; they found that the invasive populations were less infected than native ones. Sánchez *et al.* (2012) examined cestode infections in *A. franciscana* and *A. parthenogenetica* in a rare case of syntopy (co-occurrence) in Aigües-Mortes wetland, France, in summer and winter 2008. The invader showed considerably reduced cestode parasite diversity, prevalence and abundance. However, both studies were based on sampling from limited time periods, and characterisation of the infections in *A. franciscana* throughout the annual life cycle has not previously been carried out anywhere in the invasive or native range of this New World species. In contrast, a recent study from two sites in southern Spain found consistently high levels of cestode infections in native *A. salina* (325 km to the east of our study site) and *A. parthenogenetica* (90 km to the northwest) throughout their annual cycle, which were partly related to the density and biomass of avian definitive hosts (Sánchez *et al.* 2013b).

The aim of the present study is to quantify the helminth infection in *A. franciscana* at a site in its invasive range throughout its annual life cycle. We also compared data on cestode infections with the abundance of waterbirds, in order to establish if low infection levels can be explained by a lack of appropriate avian hosts. We discuss our results in relation to the findings of the previous studies of helminths in native and invasive *Artemia*.

Materials and Methods

Samples of *Artemia franciscana* were collected from a pre-crystallization pond (local name 13N, 3.67 ha, 36°35.799'N, 6°12.597'W) of La Tapa saltern (400 ha) near El Puerto de Santa María, Cádiz Province (Andalusia), on the Atlantic coast of Spain, between December 2006 and September 2007 (1 December 2006, 19 February, 26 April, 26 June and 18 September 2007). The salinity of this pond ranged between 86 and 209 g l⁻¹ during the study (Unión Salinera de España, S.A., unpublished data). This saltern is used for industrial salt pro-

duction (Hortas 1997) and is in the Cádiz Bay Natural Park. All waterbirds present at La Tapa salterns were counted on a monthly basis throughout 2006 and 2007 (Consejería de Medio Ambiente 2007). The study pond consistently held high numbers of waterbirds (personal observations).

Sampling was carried out from close to the shoreline with a 0.5 mm mesh sweep net, and the brine shrimps were preserved in 80% alcohol. From each sample, 200 adult individuals were examined for parasites, except for September 2007 when only 149 individuals were present in the sample. The brine shrimps were prepared as temporary glycerol mounts and examined under the stereomicroscope or compound microscope. If the identification of the larval cestodes (cysticercoids) was not possible at this stage, they were isolated by preparatory needles and mounted in Berlese's medium to facilitate observations on the morphology of rostellar hooks. Identification of cysticercoids followed Robert and Gabrion (1991), Georgiev *et al.* (2005) and Vasileva *et al.* (2009). Larval nematodes were studied in temporary glycerol mounts.

In order to characterise the parasite infections, we measured the prevalence (P: proportion of individuals infected), the mean abundance (MA: number of cysticercoids averaged for all brine shrimp individuals) and the mean intensity (MI: number of cysticercoids averaged for all infected brine shrimp individuals) for the overall parasite infection and for each parasite species (see Bush *et al.* 1997 for detailed definitions of infection descriptors).

Statistical analyses were conducted using Statistica 6.0 (StatSoft 2001).

Results

Larvae of five helminth species were recorded in *A. franciscana* in La Tapa saltern. These included cysticercoids of four cestode species, three of which are parasitic as adult in greater flamingos *Phoenicopterus roseus*: *Flamingolepis liguloides* (Fig. 1a,b) and *F. flamingo* (Skrjabin, 1914) (Fig. 1c) of the family Hymenolepididae, and *Gynandrotaenia stammeri* Fuhrmann, 1936 (Fig. 1e,f) of the family Progynotaeniidae. *Eurycestus avoceti* Clark, 1954 of the family Dilepididae (Fig. 1d) is a parasite of avocets *Recurvirostra avosetta*, black-winged stilts *Himantopus himantopus* and Kentish plovers *Charadrius alexandrinus*. In addition, third-stage larvae of spirurid nematodes (referred to from hereon as "Acuariinae gen. sp.") were found (Fig. 1g, h). These were a morphologically homogeneous series (probably representing one species) and exhibited the presence of muscular and glandular portions of the oesophagus (as in the order Spirurida), lateral triangular pseudolabia, elongate buccal cavity and cordons arising from the bases of pseudolabia (as in the subfamily Acuariinae of the family Acuariidae) (Chabaud 1975, Anderson 2000).

The overall infection rate was low, with total prevalence 5.9% and values of the prevalence of the individual parasite species varying between 0.2 and 3.2%; the mean abundance

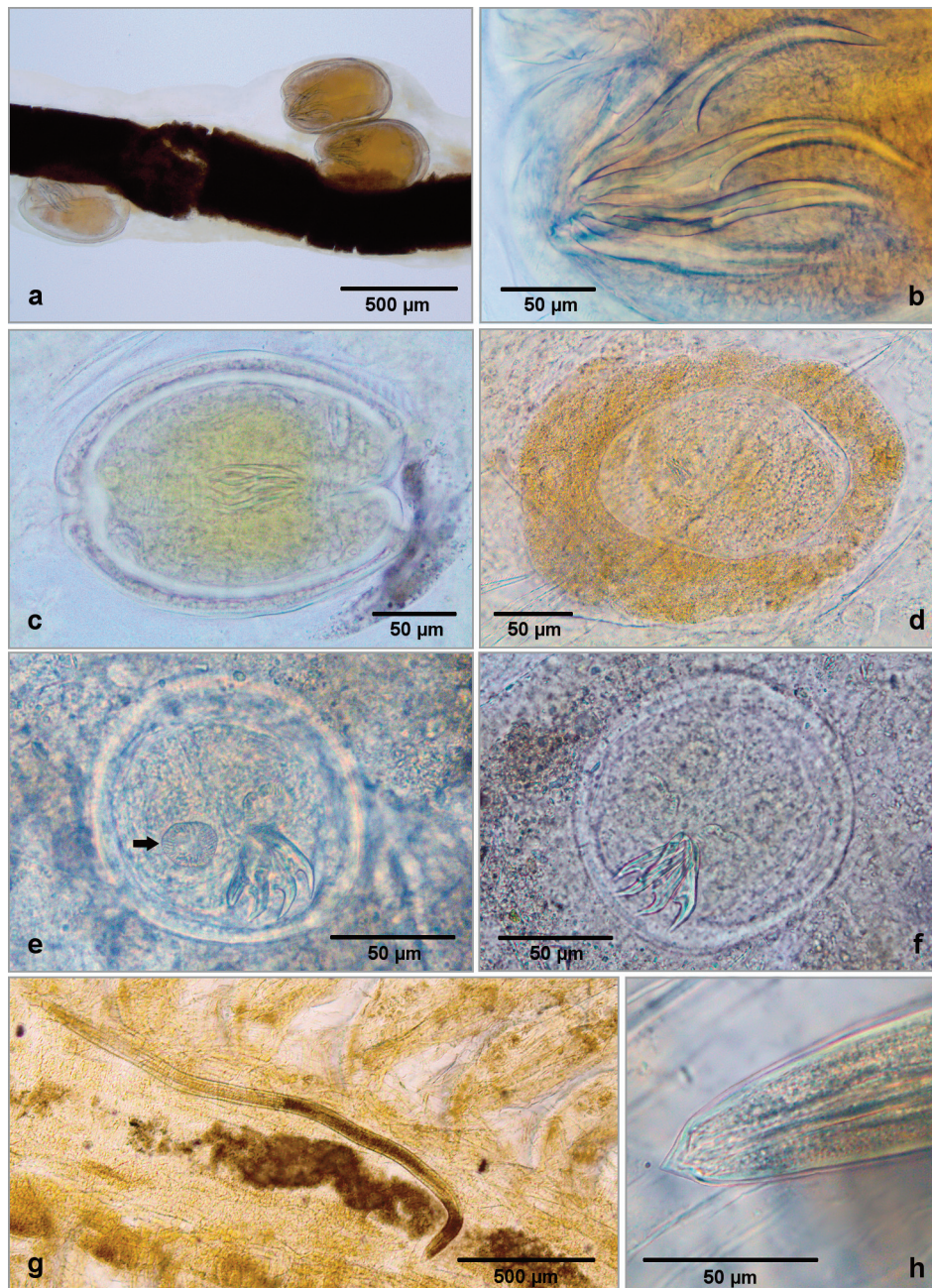


Fig. 1. Larvae of helminth parasites recorded in *Artemia franciscana* from La Tapa: **a** – *Flamingolepis liguloides* (Hymenolepididae), three cysticercoids in the abdomen of *Artemia*. **b** – *F. liguloides*, a detail demonstrating the shape of the rostellar hooks. **c** – *Flamingolepis flamingo* (Hymenolepididae), a cysticercoide in the haemocoel of *Artemia*. **d** – *Eurycestus avoceti* (Dilepididae), a cysticercoide in a thoracopod of *Artemia*. **e, f** – *Gynandrotaenia stammeri* (Progynotaeniidae), cysticercoids in the haemocoel of *Artemia*; note armament of suckers (arrow). **g** – Nematode larva of the subfamily Acuariinae in the haemocoel of *Artemia*, general view. **h** – Nematode larva of the subfamily Acuariinae, cephalic end, lateral view, demonstrating the triangular pseudolabium typical for the family

was also low (Table I). The number of the helminth species varied between one in April and four in February and September. The only parasite species recorded in all the samples was *E. avoceti*. The most prevalent and abundant parasite was *F. flamingo*; however, its prevalence and mean abundance in the samples studied did not exceed 8.5% and 0.090 ± 0.021 , respectively (maximum values from February).

In total, 65 helminth larvae were found: 31 of *F. flamingo*, 24 of *E. avoceti*, 4 of *F. liguloides*, 3 of *G. stammeri* and 3 of Acuariinae gen. sp. Out of 56 infected brine shrimps, 52 contained a single helminth species. Multiple infections were combinations of *F. flamingo* and *E. avoceti* recorded in one brine shrimp collected in December and in three sampled in February. One of the latter host individuals exhibited the

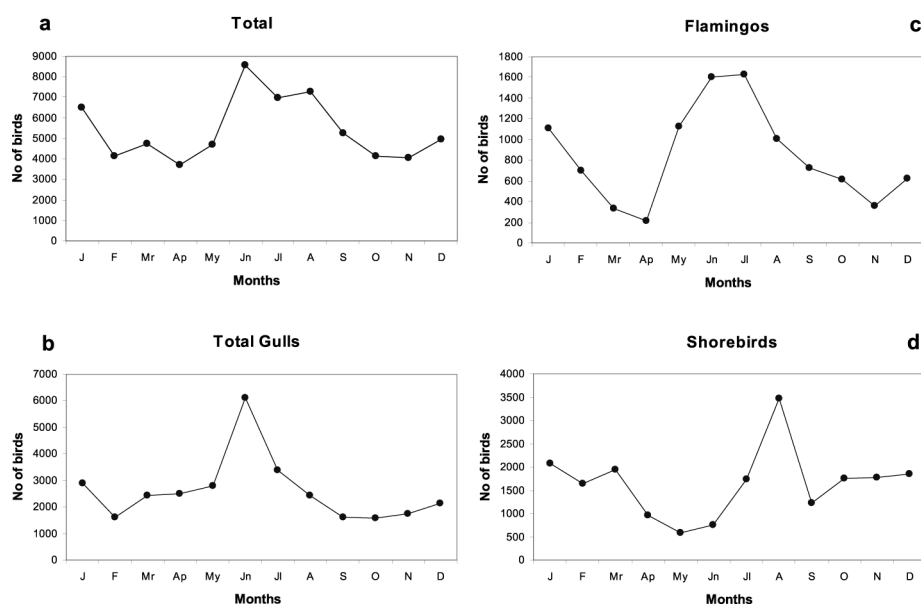


Fig. 2. Seasonal variation of waterbird numbers in La Tapa salt pans in 2006–2007, presenting mean values for two years: **a** – Total number of birds. **b** – Number of gulls. **c** – Number of flamingos. **d** – Number of shorebirds. See Appendix I for details of the abundance of individual species

highest intensity of infection recorded, i.e. two cysticercoids of *F. flamingo* and two of *E. avoceti*.

Pairwise comparisons of all the values of mean abundance and mean intensity between seasonal samples were performed using Mann-Whitney U Tests. The only significant difference detected was that between the values of the total mean abundance in February (month of peak abundance) and April 2007 ($U = 17499$, $p = 0.030$). No significant differences in mean intensity were detected. The same test was applied to compare helminth infections between male and female brine shrimps as well as between three size classes of *Artemia* individuals (<6 mm long, 6–8 mm long and 8–10 mm long). No significant differences of the infections between sexes or size classes were revealed, although our statistical power was very low.

Of the infected 33 female brine shrimps, 21 had eggs in the ovisac (63.6%). For the non-infected females ($n = 407$), this proportion was only 34.4%. Comparing brine shrimps with eggs, there was no significant difference between the number of eggs in infected and non-infected female individuals, i.e. no evidence for reduced fecundity due to helminth infections. However, age and seasonal differences could be confounding factors here (e.g. infected individuals may tend to be older).

Waterbird counts showed that the avian community consisted of 49 species, many of them represented by substantial numbers and biomass (Appendix I). Waterbirds were abundant throughout the year, with mean total counts varying between 3000 and 9000 (Fig. 2a). The salt pans are inhabited mainly by gulls (av. 2600 individuals/month), shorebirds (av. 1650 individuals/month) and flamingos (*Phoenicopterus roseus*) (av. 840 individuals/month). These groups, known to be definitive hosts of cestodes using native brine shrimps as intermediate hosts, were all abundant throughout the year but

with maxima in summer months (Fig. 2b–d). The abundance of grebes and common shelducks, also known as hosts of cestodes developing in *Artemia*, was much lower (Appendix I).

Discussion

The invasion of *Artemia franciscana* in the Mediterranean Region and in Asia (Amat *et al.* 2005, 2007; Vikas *et al.* 2012) has probably led to the most important biodiversity loss in hypersaline ecosystems to date. Previous studies indicating the possible role of helminth parasites as a factor promoting the competitive success of *A. franciscana* in the course of its invasion in the Mediterranean have been based on few samples and seasons (Georgiev *et al.* 2007; Sánchez *et al.* 2012). Research considering the whole annual cycle provides more reliable results, as helminth infections often vary seasonally. The present study, conducted during a full year, is the most exhaustive investigation of parasite infection in *A. franciscana* to date. In contrast to similar studies of native *Artemia* (Sánchez *et al.* 2013b), parasite infection was consistently low throughout the year, suggesting that parasites have a crucial role in the competitive success of this highly invasive species.

The cestode community detected in *A. franciscana* inhabiting La Tapa salt pans has lower species richness (4 species) than reported for one *A. franciscana* population from Portugal (6 species), but higher richness than recorded at other sites from Spain (1–2 species) and France (2 species), albeit over shorter periods (Georgiev *et al.* 2007; Vasileva *et al.* 2009; Sánchez *et al.* 2012). In contrast, cestode communities in *A. parthenogenetica* and in *A. salina* studied over the whole annual cycle consisted of 9 cestode species each (Sánchez *et al.* 2013b).

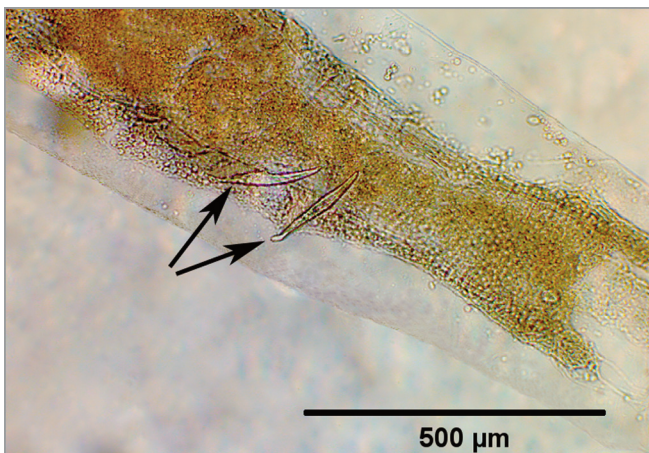


Fig. 3. Abdomen of *Artemia franciscana* containing isolated rostellar hooks (arrows) originating from a dead cysticercoid of *Flamingolepis liguloides*

Sánchez *et al.* (2013b) found the cestode infection rates in native *A. parthenogenetica* and *A. salina* to be high. Throughout the year, 4–45% of *A. parthenogenetica* in Odiel salt pans and 27–72% of *A. salina* from Salinas de Cerrillos were infected with cestodes (compared to 0.5–13% of *A. franciscana* from La Tapa). The overall mean abundance of cysticercoids varied between 0.05 and 0.81 (av. 0.35) in *A. parthenogenetica* and between 0.44 and 1.32 (av. 0.90) in *A. salina* (Sánchez *et al.* 2013b). This contrasts with mean abundance of helminth parasites in *A. franciscana* ranging between 0.005 and 0.155 (av. 0.068), i.e. 5–13 times smaller than in the native species.

Our results also demonstrate that the most prevalent and abundant cestode species in native brine shrimps, *Flamingolepis liguloides*, has consistently lower prevalence and abundance in the invasive *A. franciscana* throughout the year. The frequent records of dead cysticercoids (Fig. 3) in *A. franciscana* (unpublished data) suggest that low prevalence of *F. liguloides* in this brine shrimp species may be related to a strong immune response by the host. For the remaining three cestode species recorded in the present study (*Flamingolepis flamingo*, *Gynandrotaenia stammeri* and *Eurycestus avoceti*), the values of prevalence and abundance are generally similar in invasive and native *Artemia* spp. (see Sánchez *et al.* 2013b).

The absence of larval *Fimbriarioides tadornae* and *Confluaria podicipina* (parasitic as adults in shelducks and grebes, respectively) at La Tapa can be explained by the absence or low abundance of the appropriate definitive hosts in the wetland studied (Appendix I). However, the same explanation is not applicable for the lack of species of the genus *Anomotaenia* (parasitic in shorebirds), *Wardium* and *Branchiopodotaeonia* (both parasitic in gulls), since the relevant host groups were abundant at La Tapa (Fig. 2b,d). This suggests variation between cestode species in their ability to adapt to the new, invasive intermediate host. Furthermore, *F. flamingo* was consistently more abundant than *F. liguloides* (Table I), the inverse of what is recorded in native brine shrimps (Sánchez

et al. 2013b), even though these species share the same final host. The absence of *A. tringae* in the present material is also surprising since this was the most prevalent and abundant cestode species in another *A. franciscana* population in a different area of Cádiz Bay (Georgiev *et al.* 2007). On this basis, we can speculate that the co-adaptation between invasive hosts and native parasites at different invaded sites has achieved different levels of development. Based on neutral genetic markers, there are important differences between the different *A. franciscana* populations found in the Iberian Peninsula (Muñoz *et al.* 2014).

The present results on parasite infection of *A. franciscana* during its entire life cycle are consistent with the previous short-term data demonstrating that the parasite infection rate is much lower in invasive than in native brine shrimp (Georgiev *et al.* 2007; Sánchez *et al.* 2012). We found no evidence of regulating effects of parasite infections on the populations of *A. franciscana*, although these are hard to detect due to the low infection rates (see also Sánchez *et al.* 2012). The lack of parasite-induced control on the host population contrasts strongly with parasite-associated modifications of appearance, behaviour and fecundity in the native *Artemia* reported previously (Thiéry *et al.* 1990; Amat *et al.* 1991a; Varó *et al.* 2000; Sánchez *et al.* 2006a, 2007, 2009b, 2012, 2013; Rode *et al.* 2013). Therefore, the differences of parasite burden in native and invasive brine shrimp may result into differential predator-prey outcomes (Sánchez *et al.* 2009a; Dunn *et al.* 2012), i.e. differential parasite-induced mortality. This, in combination with the reduced fecundity of infected native brine shrimps (Amat *et al.* 1991a; Sánchez *et al.* 2012), explains the competitive success of *A. franciscana* in the Mediterranean. Thus, the replacement of the native populations of *Artemia* spp. by the invasive species can be partially explained by a competition mediated by parasites/predators through a differential impact on host fitness.

The acuariid nematodes recorded in the course of the present study could not be identified to genus due to the relatively simple morphology of larvae, which does not resemble that of adults, as well as the limited previous studies, which are insufficient to link larval and adult stages of species in this family on the basis of morphology. Though rarely, we have also recorded similar nematode larvae in *A. parthenogenetica* from Odiel salt pans (Huelva Province) and from *A. salina* from Salinas de Cerrillos (Almería Province) (unpublished data). Genera of the Acuariinae have been differentiated on the basis of the morphology of adults, which typically occur in the stomach (under the gizzard lining), proventriculus or oesophagus of birds (Skryabin *et al.* 1965; Chabaud 1975). Until now, no nematode species has been reported to utilise *Artemia* spp. as intermediate host in its life cycle (Anderson 2000). On the basis of the species diversity and abundance of the potential definitive hosts in hypersaline wetlands, it can be speculated that the larval nematodes may belong to the genera *Decorataria* Sobolev in Skryabin, Shikhobalova and Sobolev, 1949 (parasitic in grebes, see Mutafchiev and Georgiev 2008a), *Chevreuria* Seurat, 1918

Table I. Prevalence (P%), mean abundance (MA \pm SE), range of intensity (I) and mean intensity (MI \pm SE) of helminth parasites in *Artemia franciscana* in La Tapa

	P%	MA \pm SE	I	MI \pm SE
December 2006 (N = 200)				
<i>Flamingolepis liguloides</i>	0.5	0.015 \pm 0.015	3–3	3.00
<i>Flamingolepis flamingo</i>	3.5	0.035 \pm 0.013	1–1	1.00
<i>Eurycestus avoceti</i>	1.5	0.015 \pm 0.009	1–1	1.00
Total	5.0	0.065 \pm 0.022	1–3	1.30 \pm 0.22
February 2007 (N = 200)				
<i>Flamingolepis liguloides</i>	0.5	0.005 \pm 0.005	1–1	1.00
<i>Flamingolepis flamingo</i>	8.5	0.090 \pm 0.021	1–2	1.06 \pm 0.06
<i>Eurycestus avoceti</i>	5.0	0.055 \pm 0.018	1–2	1.10 \pm 0.10
<i>Gynandrotaenia stammeri</i>	0.5	0.005 \pm 0.005	1–1	1.00
Total	13.0	0.155 \pm 0.032	1–4	1.19 \pm 0.12
April 2007 (N = 200)				
<i>Eurycestus avoceti</i>	0.5	0.005 \pm 0.005	1–1	1.00
Total	0.5	0.005 \pm 0.005	1–1	1.00
June 2007 (N = 200)				
<i>Flamingolepis flamingo</i>	1.5	0.015 \pm 0.009	1–1	1.00
<i>Eurycestus avoceti</i>	3.5	0.035 \pm 0.013	1–1	1.00
Total	5.0	0.050 \pm 0.015	1–1	1.00
September 2007 (N = 149)				
<i>Flamingolepis flamingo</i>	2.0	0.020 \pm 0.011	1–1	1.00
<i>Eurycestus avoceti</i>	0.7	0.013 \pm 0.013	2–2	2.00
<i>Gynandrotaenia stammeri</i>	1.3	0.013 \pm 0.009	1–1	1.00
Acuariinae gen. sp.	2.0	0.020 \pm 0.011	1–1	1.00
Total	6.0	0.067 \pm 0.023	1–2	1.11 \pm 0.10
Overall infection (N = 949)				
<i>Flamingolepis liguloides</i>	0.2	0.004 \pm 0.004	1–3	2.00 \pm 1.00
<i>Flamingolepis flamingo</i>	3.2	0.033 \pm 0.006	1–2	1.03 \pm 0.03
<i>Eurycestus avoceti</i>	2.3	0.025 \pm 0.006	1–2	1.09 \pm 0.06
<i>Gynandrotaenia stammeri</i>	0.3	0.003 \pm 0.002	1–1	1.00
Acuariinae gen. sp.	0.3	0.003 \pm 0.002	1–1	1.00
Total	5.9	0.068 \pm 0.010	1–4	1.16 \pm 0.07

(parasites of stilts and avocets, see Smogorzhevskaya 1990), *Syncuaria* Gil'bert, 1927 (parasites of storks and ibises, see Mutafchiev and Georgiev 2008b), *Sexansocara* Sobolev and Sudarikov, 1939 (parasitic in ospreys, see Baruš *et al.* 1978), *Voguracuaria* Wong and Anderson, 1993 (parasitic in dunlins, see Wong and Anderson 1993) or *Echinuria* Soloviev, 1912 (parasitic in grebes, gulls and ducks, see Baruš *et al.* 1978; Smogorzhevskaya 1990). All these genera have unknown or poorly-studied life cycles and undescribed (or inadequately characterized) morphology of larval stages (Skryabin *et al.* 1965; Anderson 2000). Other potential nematode parasites in birds in our study area include the genera *Cosmocephalus* Molin, 1858 (known from grebes, gulls and redshanks, see Mutafchiev *et al.* 2010), *Skrjabinocerca* Shikhobalova, 1930 (parasitic of plovers and sandpipers, see Wong and Anderson 1993), *Skrjabinocara* Kurashvili, 1940 (parasitic in cormorants, see Smogorzhevskaya 1990), *Skrjabinoclava* Sobolev, 1943

(parasitic of turnstones, curlews and sandpipers, see Anderson and Wong 1992) or *Desportesius* Chabaud and Campana, 1949 (mostly parasitic in herons, see Wong and Anderson 1986). However, their larvae are characterized by morphology (as described by Chabaud 1950; Wong and Anderson 1982, 1987; Wong *et al.* 1989; Bartlett *et al.* 1989) differentiating them from the nematodes we have recorded here. The elucidation of the diversity of the larval nematodes parasitizing *Artemia* spp. therefore requires further studies.

The low vulnerability to native parasites of *A. franciscana* can have a wide range of direct and indirect ecological and evolutionary consequences. Given that cestodes are parasites with complex life cycles using food webs for transmission, we expect effects in consumer–resource interactions at different trophic levels. *Artemia* is the dominant grazer in hypersaline ecosystems and it can control phytoplankton abundance (Mehbibi 2010). Therefore, the lack of regulation of *A. francis-*

cana population density by parasites (compared with native *Artemia* populations) may have an impact on phytoplankton density, with potential cascading effects. On the other hand, the density of *A. franciscana* is more likely to become limited by reduced phytoplankton abundance. This is supported by our unpublished observations of *A. franciscana* from the study area affected by Black Spot Disease, which is indicative of dietary deficiency. Similar observations have been reported in *A. franciscana* from Great Salt Lake in the native range, under unusually low phytoplankton abundance (Lavens and Sorgeloos 1996; Belovsky *et al.* 2011). We also expect an impact of the invasion on avian communities. Many species of shorebirds are highly dependent on salt pans and *Artemia* as a food source (Sánchez *et al.* 2005, 2006b), and prey selection experiments have shown that they selectively feed on infected *Artemia*, which are more accessible and profitable from an energetic point of view (Sánchez *et al.* 2009a, 2009b). Therefore waterbirds may have lower foraging intake rates when feeding in salt ponds invaded by *A. franciscana*. Future studies should consider the broader ecological consequences of establishment and spread of the invasive *A. franciscana*.

Finally, it would be interesting to explore the potential role of microorganisms on the successful establishment of *A. franciscana*. Contrary to helminth parasites, which are not present in dormant brine-shrimp eggs ("cysts", i.e. the life-cycle stage in which *A. franciscana* was introduced), several bacteria, viruses and fungi (mainly microsporidia) have been identified in *Artemia* cysts and nauplii from the field and from commercial samples (Austin and Allen 1982; Igarashi *et al.* 1989). Microsporidia in particular can be highly prevalent in invasive populations of *A. franciscana* (Rode *et al.* 2013) and provoke high levels of mortality in native *Artemia* populations (personal observation). The potential introduction of novel parasites together with *A. franciscana* into the introduced range and their impact on native host populations should also be assessed.

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Appendix I. Mean values of monthly waterbird counts at the La Tapa salterns for the period from January 2006 to December 2007 and their conversion into biomass (g) based on Snow *et al.* (1998)

Bird groups and species	Mean count	Mean biomass
Grebes (Podicipedidae)		
Great Crested Grebe (<i>Podiceps cristatus</i>)	0.04	43.77
Cormorants (Phalacrocoracidae)		
Great Cormorant (<i>Phalacrocorax carbo</i>)	70.33	161766.67
Hérons and Egrets (Ardeidae)		
Cattle Egret (<i>Bubulcus ibis</i>)	4.13	1443.75
Little Egret (<i>Egretta garzetta</i>)	8.50	3825.00
Grey Heron (<i>Ardea cinerea</i>)	21.71	33571.94
Storks (Ciconiidae)		
White Stork (<i>Ciconia ciconia</i>)	0.46	1535.42
Ibises and Spoonbills (Threskiornithidae)		
Common Spoonbill (<i>Platalea leucorodia</i>)	32.00	49440.00
Flamingos (Phoenicopteridae)		
Greater Flamingo (<i>Phoenicopterus roseus</i>)	836.79	2614973.96
Ducks and Geese (Anatidae)		
Common Shelduck (<i>Tadorna tadorna</i>)	3.13	3476.56
Common Teal (<i>Anas crecca</i>)	0.04	13.54
Mallard (<i>Anas platyrhynchos</i>)	5.71	6065.10
Northern Shoveler (<i>Anas clypeata</i>)	7.33	4620.00
Ospreys (Pandionidae)		
Osprey (<i>Pandion haliaetus</i>)	0.67	1166.67
Rails (Rallidae)		
Eurasian Coot (<i>Fulica atra</i>)	3.04	2433.33
Gulls and Terns (Laridae)		
Mediterranean Gull (<i>Larus melanocephalus</i>)	0.04	10.67
Black-headed Gull (<i>Chroicocephalus ridibundus</i>)	118.92	34188.54
Slender-billed Gull (<i>Larus genei</i>)	11.08	3175.38
Audouin's Gull (<i>Larus audouinii</i>)	5.214	010.42
Lesser Black-backed Gull (<i>Larus fuscus</i>)	101.46	79873.07
Yellow-legged Gull (<i>Larus michahellis</i>)	2098.25	2486426.25

Caspian Tern (<i>Sterna caspia</i>)	0.54	367.25
Sandwich Tern (<i>Sterna sandvicensis</i>)	26.25	6431.25
Common Tern (<i>Sterna hirundo</i>)	3.33	433.33
Little Tern (<i>Sternula albifrons</i>)	43.33	2426.67
Whiskered Tern (<i>Chlidonias hybridus</i>)	0.25	22.88
Black Tern (<i>Chlidonias niger</i>)	95.17	5900.33
Shorebirds		
Oystercatchers (Haematopodidae)		
Eurasian Oystercatcher (<i>Haematopus ostralegus</i>)	1.38	742.50
Stilts and Avocets (Recurvirostridae)		
Black-winged Stilt (<i>Himantopus himantopus</i>)	137.92	24825.00
Eurasian Avocet (<i>Recurvirostra avosetta</i>)	375.54	103273.96
Stone curlews (Burhinidae)		
Eurasian Stone Curlew (<i>Burhinus oediconemus</i>)	38.83	18057.50
Plovers (Charadriidae)		
Common Ringed Plover (<i>Charadrius hiaticula</i>)	75.58	4837.33
Kentish Plover (<i>Charadrius alexandrinus</i>)	91.46	4344.27
Grey Plover (<i>Pluvialis squatarola</i>)	34.21	9920.42
Sandpipers and Allies (Scolopacidae)		
Red Knot (<i>Calidris canutus</i>)	0.33	59.17
Sanderling (<i>Calidris alba</i>)	50.00	4012.50
Little Stint (<i>Calidris minuta</i>)	54.63	1843.59
Curlew Sandpiper (<i>Calidris ferruginea</i>)	154.71	12415.34
Dunlin (<i>Calidris alpina</i>)	283.96	13488.02
Ruff (<i>Philomachus pugnax</i>)	0.04	7.60
Black-tailed Godwit (<i>Limosa limosa</i>)	195.50	60116.25
Bar-tailed Godwit (<i>Limosa lapponica</i>)	46.83	15455.00
Whimbrel (<i>Numenius phaeopus</i>)	0.75	406.88
Eurasian Curlew (<i>Numenius arquata</i>)	1.21	1069.38
Green Sandpiper (<i>Tringa ochropus</i>)	0.08	7.79
Common Redshank (<i>Tringa totanus</i>)	91.67	10656.25
Common Greenshank (<i>Tringa nebularia</i>)	2.96	591.67
Common Sandpiper (<i>Actitis hypoleucos</i>)	0.58	35.00
Ruddy Turnstone (<i>Arenaria interpres</i>)	9.42	1106.46
Phalarope (<i>Phalaropus tricolor</i>)	0.04	0.01