

High prevalence of cestodes in *Artemia* spp. throughout the annual cycle: relationship with abundance of avian final hosts

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Received: 7 November 2012 / Accepted: 7 February 2013 / Published online: 6 March 2013
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Abstract Brine shrimp, *Artemia* spp., act as intermediate hosts for a range of cestode species that use waterbirds as their final hosts. These parasites can have marked influences on shrimp behavior and fecundity, generating the potential for cascading effects in hypersaline food webs. We present the first comprehensive study of the temporal dynamics of cestode parasites in natural populations of brine shrimp throughout the annual cycle. Over a 12-month period, clonal *Artemia parthenogenetica* were sampled in the Odiel marshes in

Huelva, and the sexual *Artemia salina* was sampled in the Salinas de Cerrillos in Almería. Throughout the year, 4–45 % of *A. parthenogenetica* were infected with cestodes (mean species richness=0.26), compared to 27–72 % of *A. salina* (mean species richness=0.64). Ten cestode species were recorded. Male and female *A. salina* showed similar levels of parasitism. The most prevalent and abundant cestodes were those infecting the most abundant final hosts, especially the Greater Flamingo *Phoenicopterus ruber*. In particular, the flamingo parasite *Flamingolepis liguloides* had a prevalence of up to 43 % in *A. parthenogenetica* and 63.5 % in *A. salina* in a given month. Although there was strong seasonal variation in prevalence, abundance, and intensity of cestode infections, seasonal changes in bird counts were weak predictors of the dynamics of cestode infections. However, infection levels of *Confluaria podicipina* in *A. parthenogenetica* were positively correlated with the number of their black-necked grebe *Podiceps nigricollis* hosts. Similarly, infection levels of *Anomotaenia tringae* and *Anomotaenia microphallos* in *A. salina* were correlated with the number of shorebird hosts present the month before. Correlated seasonal transmission structured the cestode community, leading to more multiple infections than expected by chance.

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Electronic supplementary material The online version of this article (doi:10.1007/s00436-013-3347-x) contains supplementary material, which is available to authorized users.

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Introduction

In the present study, we report on the variation in annual prevalence of different cestode parasites in natural populations of brine shrimp (their intermediate host) throughout the annual cycle and consider the density and biomass of their final hosts (waterbirds) as potential drivers of this variation. It is widely

assumed from empirical and theoretical evidence that host density plays a major role in determining patterns of parasite abundance (Roberts et al. 2002). Most of the data supporting this assumption come from directly transmitted parasites. However, for parasites with complex life cycles, the dynamics of the relationships between a parasite and its hosts are harder to resolve. An expected pattern is that the prevalence of infection in a downstream host should increase with the density of infected upstream hosts. For instance, Hechinger and Lafferty (2005) found that areas with a high density and diversity of final host birds had a high prevalence and diversity of larval trematodes in snails, the downstream host in the trematode life cycle. However, this prediction is complicated if, as in our system (Sánchez et al. 2009a), final hosts selectively remove infected intermediate hosts through predation (Lafferty 1992). In such a case, even though infection rates might increase with bird density, loss rates of the parasite due to disproportionate predation on infected brine shrimp should also increase with bird density.

Brine shrimp are keystone taxa in hypersaline ecosystems where they are vital prey for shorebirds, flamingos, gulls, grebes, and other waterbirds (Sánchez et al. 2006a, 2007a; Varo et al. 2011). The native taxa present in the Mediterranean region are the sexual species *Artemia salina* (L.) and a series of clonal populations often collectively cited as *Artemia parthenogenetica* Barigozzi (see Muñoz et al. 2008, 2010). These brine shrimp in the Mediterranean are parasitized by at least 12 species of cestode, which use the shrimp as intermediate hosts and different groups of waterbirds as final hosts (Georgiev et al. 2005, 2007; Vasileva et al. 2009). Cestode eggs pass with bird feces and infect brine shrimp. The cestodes influence the behavior and coloration of the infected brine shrimp, causing them to spend more time at the water surface and become bright red, thus increasing their exposure to avian predators (Sánchez et al. 2006b, 2007b, 2009a, 2013). They also affect the lipid content of shrimp (Amat et al. 1991a; Sánchez et al. 2009b). This increases the caloric content of infected shrimp, which may benefit predators. Furthermore, cestodes reduce the fecundity of the shrimp (Amat et al. 1991a; Varó et al. 2000). By influencing the survival and reproductive rates of the dominant grazer in hypersaline systems, cestodes have the potential to cause strong cascading effects at other trophic levels (e.g., by releasing algae from grazing pressure).

There have been previous studies on seasonal variation in the cestode *Flamingolepis liguloides*, which is particularly abundant in native *Artemia* and has an especially large and visible cysticercoid (Fig. 1), making it easy to quantify (Gabrion et al. 1982; Thiéry et al. 1990; Amat et al. 1991b; Mura 1995; Amarouyache et al. 2009). These studies demonstrate that infection rates are highly dynamic, but the reasons for this temporal variability remain poorly understood. Furthermore, previous studies have not considered



Fig. 1 Cysticercoids of *F. liguloides* in the abdomen of *A. parthenogenetica*

the species-rich parasite complex occurring in brine shrimp and the possible interactions among its members.

We sampled every other month to identify the temporal dynamics of different cestode species in a population of *A. salina* and another of *A. parthenogenetica* throughout the annual cycle. Given the strong seasonal patterns in key environmental variables in salt ponds, such as temperature and the density of different waterbirds, we predicted that temporal variation might structure the cestode community (as measured by multiple infections). We also predicted that the most common cestode species in brine shrimp would be those that used the most common bird species as final hosts. Finally, we predicted that temporal variation in cestodes in *Artemia* would track changes in abundance of their final host birds, especially when taking into account time lags expected from the time required for development of cestode larvae in the intermediate host.

Materials and methods

Artemia sampling and cestode quantification

Samples of adult *A. parthenogenetica* were collected every 2 months from October 2002 to August 2003 ($n=500$ per sampling) in a secondary evaporation pond (E18, 17.2 ha, salinity range over 2001=58.6–118.9 g l^{-1}) at the Odiel Saltpans (Southwest Spain, 37°15'29"N, 6°58'25"W, see Sánchez et al. 2006a, c for a detailed description of the study area). This diploid, clonal population has been studied genetically and belongs to a set of obligate parthenogenetic lineages often grouped under the binomen *A. parthenogenetica* (see Muñoz et al. 2010).

A. salina were sampled every 2 months between October 2006 and October 2007 at Salinas de Cerrillos in Almería

Province (36°42'N 02°40'W), a complex of salt pans abandoned in 1988 and protected as a Natural Landscape by the Andalusian government (Viada 1998). *A. salina* shows extremely high genetic divergence across the Mediterranean region, and this Almerian population has no overlap in haplotypes with other known Spanish populations (Muñoz et al. 2008). The precise location of collection within the salt pan complex was chosen according to the availability of *Artemia* on each collection visit. From each sample, 200 *Artemia* adults were assessed, except for August 2007 when only 188 individuals were present in the sample. The conductivity was measured at each collection point with a WTW 340i Multimeter (range from 79 mS/cm on 22 February 2007 to 219 mS/cm on 22 August 2007).

Within a given salt pond at Odiel, there is no consistent difference between the infection levels of *Artemia* collected at the edge or in the middle of the pond (C. Matesanz, M.I. Sánchez and A.J. Green, unpublished data). All *Artemia* samples were collected from close to the shoreline with a 0.5-mm mesh sweep net and preserved in 80 % alcohol. They were later prepared as temporary glycerol mounts and examined under a stereomicroscope or compound microscope. If the identification of the cysticercoids was not possible at this stage, cysticercoids were isolated and mounted in Berlese's medium to facilitate observations on the morphology of rostellar hooks. Identification of cysticercoids followed Georgiev et al. (2005) and Vasileva et al. (2009). We measured the prevalence (P: proportion of individuals infected), the mean abundance (MA: number of cysticercoids averaged for all studied *Artemia* individuals), and the mean intensity (MI: number of cysticercoids averaged for all infected *Artemia* individuals) for total parasite infection and for each cestode species (see Bush et al. 1997 for detailed definitions of infection descriptors). We also measured species richness (i.e., the number of cestode species present in a given *Artemia* individual).

Bird data

Both study areas are protected as wetlands of international importance under the Ramsar Convention, principally owing to their value for waterbirds. The avian final hosts for each cestode species were determined from available literature [see Electronic supplementary material (ESM) Table S1]. All waterbirds present at Salinas de Cerrillos were counted on a monthly basis concurrent with *A. salina* sampling from August 2006 until October 2007 (see ESM Table S3 for summary). Bird counts were conducted for the hypersaline part (387 ha) of the wetland complex (total 497 ha) that is potential habitat for brine shrimp. Unfortunately, waterbird census data for the Odiel salt pans were not available for the period when brine shrimp were sampled. However, detailed monthly census data were present for a later 5-year period (2005–2009, see

ESM Table S2 for summary). These data were relatively consistent from year to year, so we assumed that they might correlate with bird abundances during our earlier parasite observations. However, because we lacked census data for the year of sampling at the Odiel salt pans, at this site we only considered seasonal patterns for the birds acting as definitive hosts for the two most prevalent cestode species in *A. parthenogenetica*: greater flamingos *Phoenicopterus ruber* and black-necked grebes *Podiceps nigricollis* (seasonal trends in shorebird abundance at Odiel were previously described by Sánchez et al. 2006c).

Bird counts might not be the best predictor of cestode transmission to brine shrimp. This is because the bird species in our study vary tremendously in body size (e.g., sandpipers vs. flamingoes). Larger hosts provide a larger resource for parasites (Hechinger et al. 2011), suggesting that host biomass density may be a better measure than host density for our purposes. The biomass of each waterbird species in each study area (including shorebirds, gulls, etc.) was calculated using the mean mass provided by Snow et al. (1997). The mean counts per salt pan for different bird species sharing the same cestode species were first converted to biomass and then summed together. In this way, the abundance of each group of cestodes in brine shrimp could be compared to the abundance of different final hosts in both numerical and biomass terms.

Statistical analyses

We assessed the significance of the seasonal differences in cestode prevalence (P) with Z tests and the differences between MA, MI, and species richness with Kruskal–Wallis tests and Mann–Whitney U tests. Nonparametric statistics were used owing to the lack of normality in the distributions of these parameters, even after transformations. Pairwise comparisons between months were performed with Mann–Whitney U tests, applying Bonferroni correction to P values (Rice 1989).

Given that brine shrimp samples were taken only on six or seven occasions per taxon, we lacked the necessary statistical power to identify if relationships between the abundance of avian hosts and cestodes were statistically significant. This is particularly true given the need for multiple testing, owing to the large number of bird and cestode species, combined with the possibility of time lags between the dynamics of avian final hosts and those of cestodes in intermediate hosts. We present statistically significant non-parametric correlation coefficients between infection parameters and monthly bird abundance, but the P values do not account for multiple testing. Spearman correlations were conducted in cases of lack of normality in the distributions of these parameters, even after transformations. All above statistical analyses were conducted using Statistica 6.0 (StatSoft 2001, Tulsa, OK).

To investigate patterns of community structure in the cestode community, we compared the observed frequency of multiple infections with the frequency expected under various null models. After Lafferty et al. (1994), we considered the roles of temporal heterogeneity in recruitment and competition by generating null models from the temporal samples and the data pooled across samples. We hypothesized that structure due to seasonality in birds would intensify cestode recruitment into a few months. This led to the prediction that the number of expected multiple species infections would be greater for the sum of the results of separate null models calculated from each temporal sample than for a single null model calculated from the pooled samples. Because the cestodes are large and castrate their brine shrimp hosts, we also predicted that competition among species would result in fewer observed multi-species infections than predicted by summing the results of null models for each temporal sample (see Lafferty et al. 1994, for analytical details).

Results

Cestodes in *A. parthenogenetica* in Odiel and their relation with birds

Of 3,000 adult *A. parthenogenetica* sampled from October 2002 to August 2003, 679 were infected with cestodes. Therefore, total prevalence was 22.6 % (Table 1). Among 1,041 cysticercoids recorded, 9 cestode species were identified. In decreasing order of relative abundance, the cestode community consisted of *F. liguloides* (parasite of flamingos, 71.6 % of all cysticercoids, Fig. 1), *Confluaria podicipina* (parasite of grebes, 15.05 %), *Eurycestus avoceti* (parasite of shorebirds, 6.71 %), *Flamingolepis flamingo*, *Anomotaenia tringae*, *Anomotaenia microphallos*, *Gynandrotænia stammeri*, and *Wardium stelloræ* (Table 1, ESM Table S1, for details of avian final hosts). In addition, one cysticercoid of an undescribed species of the family Progynotaeniidae (*Gynandrotænia* sp.) was recorded.

Seasonal patterns in infection varied greatly between cestode species (Table 1). Only *F. liguloides* and *E. avoceti* were recorded in all months. *F. liguloides* was the most prevalent species in all the months except in October, showing highest prevalence in June (43.0 %) and lowest in December (1.6 %). *C. podicipina* was the most abundant parasite in October, with a maximum prevalence in October (11.6 %) and a minimum in June (0 %). For all cestodes combined, mean abundance and mean intensity varied significantly between months (Table 1). Post hoc comparisons (Mann–Whitney *U* tests) showed that mean abundance was higher in August and June than all other months, and lower in December than all other months except April. Mean

intensity was higher in August than in October and December. Mean abundance and mean intensity for individual cestode species also varied with sampling date. Seven species showed significant differences between months in mean abundance, but only one (*F. liguloides*) differed in mean intensity (Table 1). Cestode species richness per host varied significantly between months, ranging between 0.04 in December and 0.56 in August (Table 1). Post hoc tests showed that August and June had significantly higher richness than other months.

In pooling the monthly samples for 3,000 shrimp, we observed 594 shrimp infected with a single cestode species, 74 with two cestode species, 11 with three cestode species, and none with four or more cestode species, leading to 107 [88.8–129.0 confidence interval (CI)] pairwise interactions among species (i.e., a shrimp with three cestode species represents three pairwise interactions). However, had the species been randomly distributed among samples, we would have expected to see only 50.3 (38.1–65.7 CI) pairwise interactions, indicating that multiple infections were more common than expected in the pooled sample, an indication that the cestode community is non-randomly structured. Calculating the expected number of pairwise interactions for each sample separately and then summing them led to 65.6 (51.9–83.7 CI) expected pairwise interactions. This increase in expected pairwise interactions suggested that correlated temporal heterogeneity in cestode recruitment was responsible for about a third of the structure in the cestode community. In August, the month of highest prevalence, 82 (67.0–99.5 CI) of the pairwise interactions were seen, though only 47.8 (32.5–62.5 CI) pairwise interactions would have been expected by chance. These results do not indicate a strong role for competitive exclusion among cestode species.

Despite being measured in different years, the biomass of a particular bird group was associated with a higher abundance of that bird group's cestodes in brine shrimp. In other words, the relative abundance of cestode taxa in *A. parthenogenetica* was positively correlated with the relative biomass of the avian final hosts in the waterbird community (Fig. 2). As a result, the cestode community was dominated by parasites of greater flamingos and of grebes that made up most of the biomass of the bird community (ESM Table S2). Over the whole annual cycle for the years 2005–2009, flamingos represented 80.2 % of the total waterbird biomass in the study area and grebes represented 6.5 %. In contrast, there was no clear relationship between cestode abundance and the numerical abundance of their final hosts (ESM Fig. S1).

Seasonal changes in flamingo abundance (ESM Fig. S2) were not correlated with the changes in abundance of the larvae of their parasites in brine shrimp, so it was not clear that patterns were dominated by transmission from flamingoes

Table 1 Prevalence (P%), mean intensity (MI±SE), mean abundance (MA±SE), and species richness (±SE) of cestodes in *A. parthenogenetica* from Odiel, October 2002 to August 2003

Parasite species (code)	Infection descriptors	October 2002, n=500	December 2002, n=500	February 2003, n=500	April 2003, n=500	June 2003, n=500	August 2003, n=500	K-W (H)	Total n=3,000
<i>Flamingolepis liguloides</i> (Fl)	P%	6.20	1.60	13.60	9.20	43.00	32.00		17.60
	MI	1.10±0.054	1.13±0.125	1.26±0.074	1.13±0.059	1.39±0.047	1.67±0.076	33.04**	1.41±0.033
	MA	0.07±0.012	0.02±0.007	0.17±0.022	0.10±0.016	0.60±0.037	0.53±0.042	465.45*	0.25±0.011
<i>Flamingolepis flamingo</i> (Ff)	P%	0.20	0.00	0.80	0.20	1.40	2.20		0.80
	MI	1.00±0.000	–	1.25±0.25	1.00±0.00	1.00±0.00	1.00±0.00	0.00	1.04±0.042
	MA	0.01±0.002	–	0.01±0.00	0.01±0.005	0.01±0.005	0.02±0.007	23.16*	0.01±0.002
<i>Wardiumstellorae</i> (Ws)	P%	0.00	0.00	0.00	0.00	0.00	0.20		0.03
	MI	–	–	–	–	–	1.00±0.00	– ^a	1.00±0.00
	MA	–	–	–	–	–	0.01±0.002	5.00	0.01±0.000
<i>Anomotaenia microphallos</i> (Am)	P%	1.80	0.00	0.00	0.00	0.00	1.20		0.50
	MI	1.11±0.111	–	–	–	–	1.00±0.00	0.00	1.07±0.067
	MA	0.02±0.007	–	–	–	–	0.01±0.005	31.95**	0.01±0.001
<i>Anomotaenia tringae</i> (At)	P%	1.40	0.00	0.00	0.00	0.20	1.60		0.53
	MI	1.00±0.00	–	–	–	1.00±0.00	1.13±0.125	0.00	1.06±0.063
	MA	0.01±0.005	–	–	–	0.01±0.002	0.02±0.007	26.89*	0.01±0.001
<i>Eurycestus avoceti</i> (Ea)	P%	0.80	0.20	0.20	0.40	1.80	9.40		2.13
	MI	1.25±0.250	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.09±0.051	3.12	1.08±0.040
	MA	0.01±0.005	0.01±0.002	0.01±0.002	0.01±0.003	0.02±0.006	0.10±0.015	156.03*	0.02±0.003
<i>Confluaria podicipina</i> (Cp)	P%	11.60	2.60	0.40	1.00	0.00	7.60		3.87
	MI	1.45±0.105	1.00±0.00	1.00±0.00	1.40±0.24	–	1.34±0.102	0.00	0.05±0.005
	MA	0.17±0.024	0.03±0.007	0.01±0.003	0.01±0.007	–	0.10±0.018	149.24*	1.35±0.064
<i>Gynandrotaenia stammeri</i> (Gs)	P%	0.20	0.00	0.00	0.00	0.20	1.40		0.30
	MI	1.00±0.00	–	–	–	1.00±0.00	1.00±0.00	0.00	1.00±0.00
	MA	0.01±0.002	–	–	–	0.01±0.002	0.01±0.005	25.07*	0.01±0.001
<i>Gynandrotaenia</i> sp. (Gsp)	P%	0.20	0.00	0.00	0.00	0.00	0.00		0.03
	MI	1.00±0.00	–	–	–	–	–	– ^a	1.00±0.00
	MA	0.01±0.002	–	–	–	–	–	5.00	0.01±0.000
TOTAL	P%	21.00	4.00	14.00	10.20	45.20	41.20		22.60
	MI	1.36±0.070	1.15±0.109	1.34±0.086	1.22±0.081	1.40±0.047	1.91±0.076	62.78**	1.52±0.033
	MA	0.29±0.029	0.05±0.011	0.19±0.024	0.12±0.018	0.63±0.038	0.81±0.056	425.64*	0.35±0.014
Species Richness		0.22±0.02	0.04±0.01	0.15±0.02	0.11±0.01	0.47±0.02	0.56±0.03	416.93*	0.26±0.02

Differences between months in intensity, abundance, and species richness are tested by Kruskal–Wallis tests. For comparisons of abundance and richness $df=5$; for comparisons of intensity $df=1-5$. See **ESM** for definitive hosts

–^a KW test not possible as the cestode species has been recorded only in 1 month

* $P<0.001$; ** $P<0.0001$

or removal of infected brine shrimp by flamingoes. In contrast, changes in the abundance of black-necked grebes (**ESM** Fig. S2) were positively correlated with changes in prevalence of *C. podicipina* in *A. parthenogenetica* ($r=0.898$, $P=0.015$, $n=6$), suggesting that transmission from grebes was stronger than differential removal of infected brine shrimp by grebes.

Cestodes in *A. salina* and their relation with birds

From a total of 1,388 *A. salina*, 1,253 cysticercoids representing nine cestode species were identified (Table 2).

They were dominated by *F. liguloides* (75.6 % of all cysticercoids) and *F. flamingo* (15 %). Two *A. salina* individuals were infected with a single cysticercoid of the undescribed *Gynandrotaenia* sp. (Table 2). The species recorded were the same as for the *A. parthenogenetica* population, except that *W. stellorae* (parasite of gulls) was recorded only in *A. parthenogenetica*, and *Fimbriarioides tadornae* (parasite of shelducks) only in *A. salina*.

The sex of *A. salina* did not affect cestode infection. Amongst the *A. salina* individuals, 51.3 % were females and 48.7 % were males ($Z=0.939$, $P=0.35$). Of these,

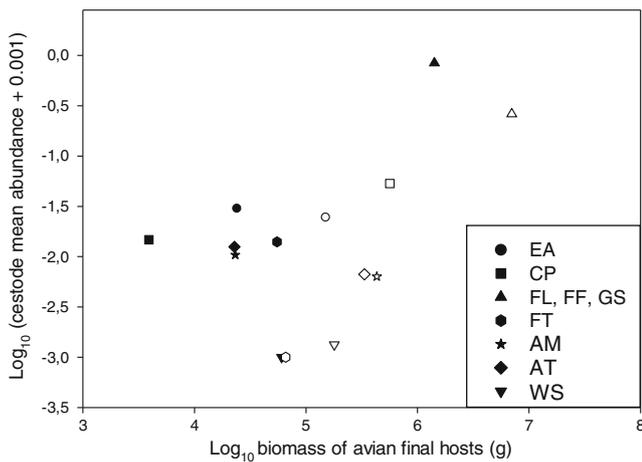


Fig. 2 Relationship between the mean abundance of cestodes and mean biomass of their avian hosts. The Y-axis presents the mean total abundance of cestodes (taken from Tables 1 and 2) parasitizing a given bird group. The X-axis presents the total waterbird biomass represented by that bird group, averaged over the whole annual cycle. *Black symbols* are for *A. salina* from Salinas de Cerrillos ($r=0.613$, $P=0.143$, $n=7$), *white ones* for *A. parthenogenetica* at Odiel ($r=0.841$, $P=0.0178$, $n=7$). See Tables 1 and 2 for the full names of cestodes and the ESM Table S1 for details of avian hosts. Without the log transformation, all points except those corresponding to flamingos are clustered close to the origin

53.2 % of females and 50.7 % of males were infected ($Z=0.691$, $P=0.49$). Males and females harbored the same species of parasites, with similar levels of infection (e.g., mean abundance and mean intensity).

Cestodes showed pronounced variation through the study period (Table 2). The total prevalence varied from 27 % (December) to 72 % (April), was highest between February and June, and was strongly influenced by changes in the prevalence of *F. liguloides* (Table 2). The lowest levels of mean abundance and intensity of infections were recorded in December and August (Table 2). Only three species were recorded in every month: *F. liguloides*, *F. flamingo*, and *E. avoceti*. There were statistically significant differences between months in the abundance of six of the nine cestode taxa (Table 2). Total cestode abundance was significantly lower in December, August, and October 2007 than in other months. Significant differences between months in intensity were only recorded for *F. liguloides* and total cestodes (Table 2). Total cestode intensity was significantly lower in August than that in other months, apart from December. There were significant differences in abundance (Mann–Whitney test, $U=14,252$, $P<0.0001$) but not intensity ($U=4,114$, $P=0.165$) of total cestodes between 26 October 2006 and 22 October 2007. Cestode species richness also varied significantly between months (Table 2). Post hoc tests showed that December, August, and October 2007 had significantly lower richness than the other four months. Overall, the mean species richness in *A. salina* was almost

two and a half times that observed for *A. parthenogenetica* at Odiel (comparison of total richness for each sample, $U=5$, $P=0.02$).

Pooling the 1,388 *A. salina* dissected monthly, we observed 573 shrimp infected with a single cestode species, 135 with two cestode species, 12 with three cestode species, 1 with four cestode species, and none with five or more cestode species, leading to 177 (154.1–202.6 CI) pairwise interactions among species. However, had the species been randomly distributed among hosts, we would have expected to see only 138.9 (118.5–162.4 CI) pairwise interactions (a shrimp with four cestode species represents six pairwise interactions), showing that multiple infections were again more common than expected based on chance, an indication that the cestode community in *A. salina* is non-randomly structured. Calculating the expected number of pairwise interactions for each sample separately and then summing them led to 155.6 (134.3–180.6 CI) expected pairwise interactions, suggesting that correlated temporal heterogeneity in cestode recruitment could explain all of the structure in the cestode community.

As at Odiel, the biomass of a particular bird group was positively correlated with a higher abundance of that bird group's cestodes in brine shrimp, although in this case the correlation was not statistically significant (Fig. 2). Again, the most abundant avian hosts in the study site were greater flamingos, representing 88.2 % of waterbird biomass (Table S3). In contrast, grebes were rare (0.24 % of waterbird biomass), coinciding with the much lower abundance of *C. podicipina* (Fig. 2). After flamingos, the next most abundant avian hosts by biomass were gulls (3.8 % of biomass) and shelducks (3.4 %). Cestodes infecting birds other than flamingos were all relatively rare (Fig. 2). There was no strong pattern between cestode abundance and the numbers of final hosts (ESM Fig. S1).

The abundance of waterbirds showed strong seasonal patterns (ESM Fig. S3), but the relationships between cestode infection descriptors (prevalence and mean abundance) and avian hosts were generally weak, suggesting no dominant effect of transmission from birds or removal of infected shrimp by birds. The exceptions were highly significant positive correlations between *A. microphallos* and the abundance of their shorebird hosts 1 month before the brine shrimp sampling, for prevalence ($r_s=0.926$, $P<0.001$), mean intensity ($r_s=0.866$, $P<0.001$), and mean abundance ($r_s=0.926$, $P<0.001$). Likewise, there were positive correlations between the mean intensity ($r_s=0.791$, $P=0.025$), prevalence ($r_s=0.709$, $P=0.054$), and mean abundance ($r_s=0.709$, $P=0.054$) of *A. tringae* and shorebird host abundance 1 month before the sampling, suggesting that transmission from shorebirds to brine shrimp influenced prevalence more than did differential removal of infected brine shrimp by birds.

Table 2 Prevalence (P%), mean intensity (MI±SE), mean abundance (MA±SE), and species richness (±SE) of cestodes in *A. salina* from Salinas de Cerrillos, October 2006 to October 2007

Parasite species (code)	Infection descriptors	October 2006, n=200	December 2006, n=200	February 2007, n=200	April 2007, n=200	June 2007, n=200	August 2007, n=188	October 2007, n=200	K-W (H)	Total n=1,388
<i>Flamingolepis liguloides</i> (Fl)	P%	52.00	22.50	53.50	63.50	55.30	31.50	27.00		43.52
	MI	1.84±0.11	1.49±0.11	1.56±0.09	1.53±0.09	1.70±0.10	1.21±0.07	1.39±0.12	27.0**	1.57±0.04
	MA	0.96±0.09	0.34±0.05	0.84±0.07	0.97±0.08	0.94±0.08	0.38±0.05	0.38±0.05	132.5**	0.68±0.027
<i>Flamingolepis flamingo</i> (Ff)	P%	16.00	5.00	19.50	21.00	8.00	3.50	9.00		11.74
	MI	1.13±0.07	1.00±0.00	1.15±0.09	1.26±0.08	1.07±0.07	1.00±0.00	1.17±0.08	8.0	1.15±0.03
	MA	0.18±0.03	0.05±0.02	0.23±0.04	0.27±0.04	0.09±0.02	0.04±0.01	0.11±0.02	58.5**	0.14±0.011
<i>Anomotaenia microphallos</i> (Am)	P%	0.50	0.00	0.50	0.00	5.30	0.00	0.50		0.94
	MI	1.00±0.00	–	1.00±0.00	–	1.00±0.00	–	1.00±0.00	0.0	1.00±0.00
	MA	0.01±0.01	–	0.01±0.01	–	0.05±0.02	–	0.01±0.01	45.8**	0.01±0.0032
<i>Anomotaenia tringae</i> At	P%	1.50	0.00	0.50	2.00	2.70	0.00	1.50		1.15
	MI	1.00±0.00	–	1.00±0.00	1.00±0.00	1.00±0.00	–	1.00±0.00	0.0	1.00±0.00
	MA	0.02±0.01	–	0.01±0.01	0.02±0.01	0.03±0.01	–	0.02±0.01	10.8	0.01±0.0032
<i>Eurycestus avoceti</i> (Ea)	P%	4.00	1.00	2.00	1.00	5.90	2.50	3.50		2.81
	MI	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	0.0	1.00±0.00
	MA	0.04±0.01	0.01±0.01	0.02±0.01	0.01±0.01	0.06±0.02	0.03±0.01	0.04±0.01	13.1	0.03±0.0044
<i>Confluaria podicipina</i> (Cp)	P%	0.50	0.00	4.50	4.50	0.00	0.00	0.00		1.37
	MI	1.00±0.00	–	1.00±0.00	1.11±0.11	–	–	–	1.1	1.05±0.05
	MA	0.01±0.01	–	0.05±0.01	0.05±0.02	–	–	–	41.1**	0.01±0.003
<i>Gynandrotaenia stammeri</i> (Gs)	P%	4.5	0.0	0.0	0.0	0.0	0.0	0.0		0.65
	MI	1.00±0.00	–	–	–	–	–	–	– ^a	1.00±0.00
	MA	0.05±0.01	–	–	–	–	–	–	53.8**	0.01±0.002
<i>Gynandrotaenia</i> sp. (Gsp)	P%	1.00	0.00	0.00	0.00	0.00	0.00	0.00		0.14
	MI	1.00±0.00	–	–	–	–	–	–	– ^a	1.00±0.00
	MA	0.01±0.01	–	–	–	–	–	–	11.9	0.01±0.001
<i>Fimbriarioides tadornae</i> (Ft)	P%	0.00	0.00	0.50	0.00	0.00	0.00	8.50		1.30
	MI	–	–	1.00±0.00	–	–	–	1.06±0.06	0.1	1.06±0.06
	MA	–	–	0.01±0.01	–	–	–	0.09±0.02	95.0**	0.01±0.003
TOTAL	P%	63.00	27.00	65.50	72.00	65.43	34.50	37.00		51.95
	MI	1.99±0.12	1.46±0.10	1.74±0.09	1.83±0.10	1.78±0.09	1.28±0.08	1.69±0.12	23.9*	1.74±0.04
	MA	1.26±0.10	0.40±0.05	1.14±0.08	1.32±0.09	1.16±0.09	0.44±0.05	0.63±0.07	172.3**	0.90±0.01
Species richness		0.80±0.05	0.29±0.03	0.81±0.05	0.92±0.05	0.77±0.05	0.38±0.04	0.50±0.05	165.7**	0.64±0.02

Differences between months in intensity, abundance, and richness are tested by Kruskal–Wallis tests. For comparisons of abundance and richness $df=6$; for comparisons of intensity $df=1-6$. See [ESM](#) for definitive hosts

–^a KW test not possible as the cestode species has been recorded only in 1 month

* $P<0.01$; ** $P<0.001$

Discussion

We observed high cestode infections throughout the year in Spanish populations of *A. salina* and *A. parthenogenetica*, with higher rates of multiple infections than expected under null models. The cestode communities were dominated by *F. liguloides*, a parasite of the final host (flamingos), which represented most of the biomass of the waterbird community. However, monthly waterbird counts at an individual salt pond were not generally good predictors of the seasonal changes in the cestodes in brine shrimp.

To our knowledge, this is the first study of dynamics of the cestode community in brine shrimp populations throughout the annual cycle. Previous studies of seasonal dynamics have only considered *F. liguloides* during a small part of the annual cycle (Gabrion et al. 1982; Thiéry et al. 1990; Amat et al. 1991b; Mura 1995; Amarouyache et al. 2009), although in many other sites adult brine shrimp are often absent for several months a year, especially during cold winter months or when temporary wetlands dry out in late summer. Among a total of 16 cestode species reported to use brine shrimp as intermediate hosts, 12 species have been

reported from the Mediterranean basin (Robert and Gabrion 1991; Georgiev et al. 2005, 2007; Vasileva et al. 2009; Redón et al. 2011). The two species not present in our samples were *Flamingolepis caroli* (another flamingo parasite, Robert and Gabrion 1991) and *Branchiopodaenia gvozdevi* (a gull parasite, Vasileva et al. 2009).

We found that correlated seasonal recruitment structured the cestode community, leading to more multiple infections than if recruitment had been homogenous throughout our samples. However, we found no evidence that competition structured the cestode community because pairwise interactions were as frequent or more frequent than expected, after accounting for temporal heterogeneity. From our data, it is not possible to determine all of the factors that led to an increase in species interactions in *A. parthenogenetica* in August, but correlated spatial heterogeneity in infection, or facilitation of one species by another are possible explanations.

Our study revealed a high (27–72 %) combined prevalence of cestodes in *A. salina* from Almería throughout the annual cycle and a somewhat lower combined prevalence (4–45 %) in clonal brine shrimp from the Odiel salt pans. The prevalence of a parasite species with a complex life cycle in intermediate host populations is often less than 5 % (Cézilly et al. 2010), but some cestode species have higher prevalences in Spanish brine shrimp populations. This, in combination with a diverse community of cestodes, leads to a high combined prevalence. In a survey of four sites in southern Spain with *A. parthenogenetica* or *A. salina* in August–September 2005, Georgiev et al. (2007) found the total prevalence of cestodes ranged from 47 to 89 %, with 45–77 % infected with *F. liguloides* alone. Our results confirm that high prevalences in *F. liguloides* can be observed throughout the year, and suggest that these cestodes are unusually abundant in their host populations in southern Spain. This is not true everywhere. In a study of the dynamics of *F. liguloides* in the brine shrimp population in French salt pans from April to October, prevalence was much lower, at 4.25 % (Gabrion et al. 1982). In brine shrimp from salt pans in the Bulgarian Black Sea coast outside the range of flamingos but frequented by other waterbirds, the prevalence of cestodes is almost zero (authors, unpublished data).

The constant release of cestode eggs from the high concentration of flamingos and other birds in our study area may explain, at least partially, the high infection levels. Both our study areas were protected because of their value for waterbirds, and hold hundreds or thousands of flamingos at any one time (ESM Figs. S2 and S3). The larger salt pans in the Odiel marshes are internationally important for birds, and during migration periods, they hold up to 20,000 shorebirds alone (Sánchez et al. 2006c).

Flamingo parasites (Fig. 2), especially *F. liguloides*, had the highest prevalence by far. The substantial biomass of greater

flamingos compared to the other waterbirds present in the study areas and their presence throughout the year (ESM Figs. S2 and S3) likely facilitate the circulation of cestodes parasitizing this bird species. This species represents the majority of waterbird biomass in many other coastal wetlands in the Iberian Peninsula (Martí and del Moral 2002; Rodríguez-Pérez and Green 2006). Our study indicates the value in considering bird biomass as an alternative to bird density in this relationship when bird species differ greatly in size. This is probably because exposure to parasites depends on feeding rates, which increase with bird size. The extraordinary prevalence of *F. liguloides* may partly be a consequence of the marked size increase of the greater flamingo population in the west Mediterranean region as a consequence of conservation measures over the past 50 years (Johnson 1997; Wetlands 2006; Rendón et al. 2008). This has coincided with a loss of wetland habitat (Green et al. 2002), leading to a strong increase (perhaps tenfold) in the density of flamingos in remaining wetlands. A lower past density of flamingos might partly explain why a much lower prevalence of *F. liguloides* was recorded in the Camargue brine shrimp population sampled in 1979 (Gabrion et al. 1982). The dominance of *F. liguloides* may also be related to the habitat use of flamingos, which prefer saline wetlands and are particularly likely to move between different wetlands suitable for brine shrimp (Amat et al. 2005a). Flamingos are also much longer-lived birds than other waterbirds in the community (Johnson 2000), and it is possible that they accumulate higher parasite loads with age, contributing to the high rates of transmission of *F. liguloides*.

Individual cestode species showed a marked seasonal pattern, with infection parameters varying significantly among months for all the species at one or both sites. In the case of *C. podicipina*, seasonal abundance at Odiel seems to be partly determined by the abundance of the final host, the black-necked grebe, which concentrates in the Odiel salt pans in autumn and early winter to moult, feeding primarily on brine shrimp (Varo et al. 2011). Similarly, we found that the infection parameters of *A. microphallos* and *A. tringae* in *A. salina* at Cerrillos were positively related to the abundance of their shorebird hosts, with a 1-month time lag likely to be explained by the development time of cysticercoids within the brine shrimp. For example, the development in *A. salina* of the cestode *B. gvozdevi* from the oncosphere to the cysticercoid takes up to 15 days under optimal salinity and temperature (Maksimova 1988).

However, for other cestode species, we did not find evidence that changes in population size of the avian host drives net seasonal changes in infection. A much longer period of sampling would be necessary to test this hypothesis (to permit time-series analysis with adequate statistical power for a large number of species). Several other factors are likely to contribute to the observed weak relationships.

Our monthly waterbird counts may not accurately reflect the abundance of avian hosts, given the continuous movements of waterbirds between sites and important changes in activity and distribution at nighttime (McNeil et al. 1992). In order for the cestodes to circulate in the bird populations, it is necessary for the birds to prey on brine shrimp. However, all the birds studied feed on a range of other items, and the relative importance of brine shrimp in the diet is likely to vary seasonally (e.g., Sánchez et al. 2005) in a manner that is not necessarily in synchrony with the fluctuations in bird numbers. In other words, changes in bird numbers may not reflect seasonal changes in the intensity of avian predation on brine shrimp. Furthermore, we did not quantify changes in the density of the brine shrimp populations within the salt ponds (but see Sánchez et al. 2006a and Varo et al. 2011 for brine shrimp dynamics at Odiel), so our data on intensity and abundance of cestodes may not accurately represent changes in the total number of cysticercoids in the whole brine shrimp population. It is also possible that some bird species selectively remove infected shrimp from the brine shrimp population (Sánchez et al. 2009a) so that periods with many birds lead to a reduction in the prevalence of patent infections. This would counteract an effect of bird density on transmission leading to the lack of pattern we observed.

Finally, seasonal changes in temperature or other environmental variables may have a stronger influence on the abundance of cysticercoids than the changes in numbers of avian hosts. Seasonal variation in temperature and salinity (see Sánchez et al. 2006a and Varo et al. 2011 for data from Odiel) may shape the infection dynamics through their effects on parasite survival or transmission success. Temperature, salinity, and dissolved oxygen can all affect cestode survival and infectivity (Chubb 1980; Sakanari and Moser 1985; Scholz 1993), and this may be especially true for the free-living cestode stages in the extreme salinities occupied by brine shrimp populations (Pietroock and Marcogliese 2003). Hence, low temperatures might partly explain why total cestode abundance reached a minimum in December for both *A. salina* and *A. parthenogenetica*. This could also be the case if infected brine shrimp suffer differential mortality under stressful conditions.

The size structure of the brine shrimp populations also undergoes seasonal changes in our study areas (Sánchez et al. 2006a; Varo et al. 2011), and this could affect parasitism. For instance, an influx of freshly hatched brine shrimp would lead to a decrease in cestode prevalence. Although we recorded significant variation between months in the body length of *A. salina* (unpublished analyses), we are confident that this does not have a major influence on the changes over time in cestode prevalence. In a generalized linear model including “month” and “sex” as factors and “length” as a continuous variable, the model estimates indicated that, when

controlling for seasonal fluctuation in *A. salina* length, the changes over time in prevalence remained very similar to those indicated in Table 2. The only change in order was that, while February and June had the second and third highest prevalence in the raw data, respectively (Table 2), these positions were reversed in the model estimates. On the other hand, the variation in size and age in a given sample might well explain our finding that multiple infections occurred more often than expected by chance. Those individual shrimp parasitized by *F. liguloides* may well tend to be relatively older, and therefore more likely to have accumulated infections by more than one cestode species. Although different cestode species can be expected to compete with each other to be consumed by their respective final hosts, we have found no evidence that competition leads to exclusion or preemption.

Implications for brine shrimp dynamics and conservation

Brine shrimp are the main primary consumer in the water column. Adults typically die as water temperatures drop, and the next generation survives the winter season in the form of diapaused eggs, which hatch in early spring (Abatzopoulos et al. 2002). Given the high combined prevalence of cestodes and their effects on fecundity of brine shrimp (Amat et al. 1991a, b; Sánchez et al. 2009a, authors unpublished data), the abundance of cestodes for 1 year may be a key determinant of the abundance of the brine shrimp population during the following year. Indeed, further research is required to establish how cestodes affect the dynamics of the brine shrimp population. Recruitment of brine shrimp individuals from the egg bank in sediments (Brendonck and de Meester 2003) or dispersal from neighboring ponds (Sánchez et al. 2007a) could help replenish the brine shrimp population after a year with high cestode prevalence.

The high prevalence of cestodes in *A. salina* and *A. parthenogenetica* populations is likely to increase the probability of invasion by the alien sexual *Artemia franciscana* Kellogg, which originates in North America. Since the 1980s, many locations in the Iberian Peninsula have been colonized by *A. franciscana* (Amat et al. 2005b), which is much less susceptible to infection by *F. liguloides* and other cestodes in its invasive range (Georgiev et al. 2005; authors' unpublished data). In most cases, after the detection of *A. franciscana* in a locality, native brine shrimp are completely outcompeted within 3 years, and release from cestode parasitism is likely to be one reason why this alien has such a strong competitive advantage.

A high prevalence and richness of cestodes indicates a high diversity and abundance of birds in this system. The recovery of flamingoes appears to be the main factor driving these high prevalences, suggesting the prevalence of cestodes in brine shrimp is a positive indicator of bird conservation efforts, as

has been suggested for larval trematodes in snails (Hechinger and Lafferty 2005). Cestodes may also make foraging easier for birds, although this remains a speculation based on the extent to which infected shrimp are more likely to be eaten than uninfected shrimp (Sánchez et al. 2009a).

Acknowledgments The Consejería de Medio Ambiente, Junta de Andalucía and Aragonésas Industrias y Energía S. A. provided permission for fieldwork. Waterbird census data were provided by the Consejería de Medio Ambiente. MIS was supported by a Juan de la Cierva postdoctoral contract. T. Stewart and anonymous referees provided comments on drafts. This work was also supported by joint research projects between CSIC and the Bulgarian Academy of Sciences (2004BG0013 and 2005BG0015), by the EC-funded project WETLANET (FP7, Programme CAPACITIES, Grant 229802), by the Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía (P07-CVI-02700) and by the Ministerio de Ciencia e Innovación (Proyecto CGL2010-16028). Any use of trade, product, website, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

- Abatzopoulos TJ, Beardmore JA, Clegg JS, Sorgeloos P (2002) *Artemia*: basic and applied biology. Kluwer Academic Publishers, Dordrecht
- Amarouayache M, Derbal F, Kara MH (2009) The parasitism of *Flamingolepis liguloides* (Gervais, 1847) (Cestoda, Hymenolepididae) in *Artemia salina* (Crustacea, Branchiopoda) in two saline lakes in Algeria. *Acta Parasitol* 54:330–334
- Amat F, Gozalbo A, Navarro JC, Hontoria F, Varó I (1991a) Some aspects of *Artemia* biology affected by cestode parasitism. *Hydrobiol* 212:39–44
- Amat F, Illescas P, Fernández J (1991b) Brine shrimp *Artemia* from Spanish Mediterranean salters parasitized by *Flamingolepis liguloides* (Cestoda, Hymenolepididae) cysticeroids. *Vie et Milieu* 41:237–244
- Amat JA, Rendon MA, Rendon-Martos M, Garrido A, Ramirez JM (2005a) Ranging behaviour of greater flamingos during the breeding and post-breeding periods: linking connectivity to biological processes. *Biol Cons* 125:183–192
- Amat F, Hontoria F, Ruiz O, Green AJ, Sánchez MI, Figuerola J, Hortas F (2005b) The American brine shrimp *Artemia franciscana* as an exotic invasive species in the Western Mediterranean. *Biol Inv* 7:37–47
- Brendonck L, De Meester L (2003) Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. *Hydrobiol* 491:65–84
- Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 83:575–583
- Cézilly F, Thomas F, Médoc V, Perrot-Minnot MJ (2010) Host-manipulation by parasites with complex life cycles: adaptive or not? *Trends Parasitol* 26:311–317
- Chubb JC (1980) Seasonal occurrence of helminths in freshwater fishes. Part III. Larval Cestoda and Nematoda. *Adv Parasitol* 18:1–120
- Gabriel C, MacDonald G, Boy V (1982) Dynamique des populations larvaires du cestode *Flamingolepis liguloides* dans une population d'*Artemia* en Camargue. *Acta Oecol* 3(2):273–293
- Georgiev BB, Sánchez MI, Green AJ, Nikolov PN, Vasileva GP, Mavrodiya RS (2005) Cestodes from *Artemia parthenogenetica* (Crustacea, Branchiopoda) in the Odiel Marshes, Spain: a systematic survey. *Acta Parasitol* 50:105–117
- Georgiev BB, Sánchez MI, Vasileva GP, Nikolov PN, Green AJ (2007) Cestode parasitism in invasive and native brine shrimps (*Artemia* sp.) as a possible factor promoting the rapid invasion of *A. franciscana* in the Mediterranean region. *Parasitol Res* 101:1647–1655
- Green AJ, El Hamzaoui M, El Agbani MA, Franchimont J (2002) The conservation status of Moroccan wetlands with particular reference to waterbirds and to changes since 1978. *Biol Cons* 104:71–82
- Hechinger RF, Lafferty KD (2005) Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proc R Soc Lond B* 272:1059–1066
- Hechinger RF, Lafferty KD, Dobson AP, Brown JH, Kuris D (2011) A common scaling rule for abundance, energetics, and production of parasitic and free-living species. *Science* 333:445–448
- Johnson AR (1997) Long-term studies and conservation of Greater Flamingos in the Camargue and Mediterranean. *Colon Waterbirds* 20:306–315
- Johnson AR (2000) An overview of the Greater Flamingo ringing program in the Camargue (southern France) and some aspects of the species' breeding biology studied using marked individuals. *Waterbirds* 23:2–8
- Lafferty KD (1992) Foraging on prey that are modified by parasites. *Am Naturalist* 140:854–867
- Lafferty KD, Sammond DT, Kuris AM (1994) Analysis of larval trematode communities. *Ecology* 75:2275–2285
- Maksimova AP (1988) A new cestode, *Wardium gvozdevi* sp. n. (Cestoda, Hymenolepididae), and its biology. *Fol Parasitol* 35:217–222
- Martí R, del Moral JC (eds.) (2002) La invernada de aves acuáticas en España. Dirección General de la Naturaleza SEO/BirdLife, Organismo Autónomo Parques Nacionales, Ministerio de Medio Ambiente, Madrid
- McNeil R, Drapeau P, Goss-Custard JD (1992) The occurrence and adaptive significance of nocturnal habits in waterfowl. *Biol Rev* 67:381–419
- Muñoz J, Gómez A, Green AJ, Figuerola J, Amat F, Rico C (2008) Phylogeography and local endemism of the native Mediterranean brine shrimp *Artemia salina* (Branchiopoda: Anostraca). *Mol Ecol* 17:3160–3177
- Muñoz J, Gómez A, Green AJ, Figuerola J, Amat F, Rico C (2010) Evolutionary origin and phylogeography of the diploid obligate parthenogen *Artemia parthenogenetica* (Branchiopoda: Anostraca). *PLoS One* 5(8):e11932
- Mura G (1995) Cestode parasitism (*Flamingolepis liguloides* Gervais, 1847) Spassky & Spasskaja, 1954 in an *Artemia* population from south-western Sardinia. *Int J Salt Lake Res* 3:191–200
- Pietroock M, Marcogliese DJ (2003) Free-living endohelminth stages: at the mercy of environmental conditions. *Trends Parasitol* 19(7):293–299
- Redón S, Amat F, Hontoria F, Vasileva GP, Nikolov PN, Georgiev BB (2011) Participation of metanauplii and juvenile individuals of *Artemia parthenogenetica* (Branchiopoda) in the circulation of avian cestodes. *Parasitol Res* 108:905–912
- Rendón MA, Green AJ, Aguilera EY, Almaraz P (2008) Status, distribution and long term changes in the waterbird community wintering in Doñana, south-west Spain. *Biol Conserv* 141:1371–1388
- Rice WR (1989) Analyzing tables of statistical tests. *Evol* 43:223–225
- Robert F, Gabriel C (1991) Cestodoses de l'avifaune camarguaise. Rôle d'*Artemia* (Crustacea, Anostraca) et stratégies de rencontre hôte-parasite. *Ann Parasit Hum Comp* 66(5):226–235
- Roberts MG, Dobson AP, Arneberg P, de Leo GA, Kreckec RC, Manfredi MT, Lanfranchi P, Zaffaroni E (2002) Parasite community ecology and biodiversity. In: Hudson PJ, Rizzoli A, Grenfell

- BT, Heesterbeek H, Dobson AP (eds) The ecology of wildlife diseases. Oxford University Press, Oxford, pp 63–82
- Rodríguez-Pérez H, Green AJ (2006) Waterbird impacts on wideongrass *Ruppia maritima* in a Mediterranean wetland: comparing bird groups and seasonal effects. *Oikos* 112:525–534
- Sakanari J, Moser M (1985) Infectivity of, and laboratory infection with, an elasmobranch cestode, *Lacistorynchus tenuis* (Van Beneden, 1858). *J Parasitol* 7:788–791
- Sánchez MI, Green AJ, Castellanos EM (2005) Seasonal variation in the diet of Redshank *Tringa totanus* in the Odiel Marshes, south-west Spain: a comparison of faecal and pellet analysis. *Bird Study* 52:210–216
- Sánchez MI, Green AJ, Castellanos EM (2006a) Temporal and spatial variation of an aquatic invertebrate community subjected to avian predation at the Odiel saltpans (SW Spain). *Arch Hydrobiol* 166:199–223
- Sánchez MI, Georgiev BB, Nikolov PN, Vasileva GP, Green AJ (2006b) Red and transparent brine shrimps (*Artemia parthenogenetica*): a comparative study of their cestode infections. *Parasitol Res* 100:111–114
- Sánchez MI, Green AJ, Castellanos EM (2006c) Spatial and temporal fluctuations in presence and use of chironomid prey by shorebirds in the Odiel saltpans, south-west Spain. *Hydrobiol* 567:329–340
- Sanchez MI, Green AJ, Amat F, Castellanos EM (2007a) Transport of brine shrimps via the digestive system of migratory waders: dispersal probabilities depend on diet and season. *Mar Biol* 151:1407–1415
- Sánchez MI, Georgiev BB, Green AJ (2007b) Avian cestodes affect the behaviour of their intermediate host *Artemia parthenogenetica*: an experimental study. *Behav Process* 74:293–299
- Sánchez MI, Hortas F, Figuerola J, Green AJ (2009a) Sandpipers select red brine shrimps rich in both carotenoids and parasites. *Ethol* 115:196
- Sánchez MI, Thomas F, Perrot-Minnot MJ, Biron DG, Bertrand-Michel J, Missé D (2009b) Neurological and physiological disorders in *Artemia* harbouring manipulative cestodes. *J Parasitol* 95:20–24
- Sánchez MI, Varo N, Matesanz C, Ramo C, Amat JA, Green AJ (2013) Cestodes change the isotopic signature of brine shrimp *Artemia* hosts: implications for aquatic food webs. *Int J Parasitol* 43:73–80
- Scholz T (1993) On the development of *Khawia baltica* Szidat, 1942 (Cestoda: Lytocestidae) a parasite of tench, *Tinca tinca* (L.). *Fol Parasitol* 40:99–103
- Snow DW, Perrins CM, Hillcoat B, Gillmor R, Roselaar CS (1997) The birds of the Western Palearctic, concise edition. Oxford University Press, Oxford, New York
- Thiéry A, Robert F, Gabrion R (1990) Distribution des populations d' *Artemia* et de leur parasite *Flamingolepis liguloides* (Cestoda, Cyclophyllidea) dans les salins du littoral méditerranéen français. *Can J Zool* 68:2199–2204
- Varó I, Taylor AC, Navarro JC, Amat F (2000) Effect of parasitism on the respiration rates of adults of different *Artemia* strains from Spain. *Parasitol Res* 86:772–774
- Varo N, Green AJ, Sánchez MI, Ramo C, Gómez J, Amat J (2011) Behavioural and population responses to changing availability of *Artemia* prey by moulting black-necked grebes, *Podiceps nigricollis*. *Hydrobiol* 664:163–171
- Vasileva GP, Redón S, Amat F, Nikolov PN, Sánchez MI, Lenormand T, Georgiev BB (2009) Records of cysticeroids of *Fimbriarioides tadornae* Maksimova, 1976 and *Branchiopodataenia gvozdevi* (Maksimova, 1988) (Cyclophyllidea: Hymenolepididae) from brine shrimps at the Mediterranean coasts of Spain and France, with a key to cestodes from *Artemia* spp. from the Western Mediterranean. *Acta Parasitol* 54:143–150
- Viada C (ed) (1998) Areas Importantes para las Aves en España. Monografía, 5. SEO/BirdLife. Madrid
- Wetlands International (2006) Waterbird population estimates, fourth edition. Wetlands International, Wageningen, the Netherlands