

Participation of metanauplii and juvenile individuals of *Artemia parthenogenetica* (Branchiopoda) in the circulation of avian cestodes

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Abstract

Adult crustaceans of the genus *Artemia* (brine shrimps) are intermediate hosts in the life cycle of cestode species parasitic in aquatic birds as their definitive hosts. However, there are no data on the role of larval and juvenile brine shrimps in the transmission of avian helminth parasites. In order to examine the possible role of early developmental stages (nauplii, metanauplii and juveniles) of *Artemia* for the circulation of avian cestodes, the natural cestode infection in the population of *Artemia parthenogenetica* from La Mata Lagoon, Mediterranean coast of Spain, was studied. Metacestodes (cysticercoids) of four cestode species were recorded in adult brine shrimps: *Flamingolepis liguloides* and *F. flamingo* (hymenolepidids parasitic in flamingos), *Confluaria podicipina* (a hymenolepidid species parasitic in grebes) and *Eurycestus avoceti* (a dilepidid species parasitic in avocets, stilts, plovers and, to lesser extent, in flamingos). No cysticercoids were found in nauplii. Two species, *F. liguloides* and *F. flamingo*, were found in metanauplii and juvenile brine shrimps. Only 36.3% of the cysticercoids of *F. liguloides* occurred in adult brine shrimps; the

remaining 63.7% were parasitic in metanauplii (39.6%) and juveniles (24.1%). Similarly, the metacestodes of *F. flamingo* were also distributed among various age groups: in adults (44.4% of cysticercoids), juveniles (27.8%) and metanauplii (27.8%). These results indicate that early developmental stages of *Artemia* have an important role for the circulation of certain parasite species. No cysticercoids of *C. podicipina* and *E. avoceti* were recorded in larval and juvenile brine shrimps. The selective infestation of larval brine shrimps with flamingo parasites is probably associated with the feeding behaviour of definitive hosts, which are filtering predators; in contrast, grebes and waders pick brine shrimps individually one by one. The possible underlying mechanism for selective infestation of metanauplii and adults by certain cestode species is associated with the size of parasite eggs, allowing only cestode species with small eggs to be ingested by larval brine shrimps.

Introduction

Several species of brine shrimps of the genus *Artemia* (Crustacea, Branchiopoda, Anostraca) are widespread in hyperhaline basins throughout the world (Triantaphyllidis et al. 1998). *Artemia* spp. participate as intermediate hosts in the life cycle of about 15 cestode species parasitising aquatic birds (for reviews, see Georgiev et al. 2005; Vasileva et al. 2009). Cestodes are known to affect the survival and fecundity of the infected brine shrimps (Amat et al. 1991b) as well as to provoke changes in their colour (Thiéry et al. 1990; Robert and Gabrion 1991; Sánchez et al. 2006), lipid metabolism (Amat et al. 1991b), general physiology (Varó et al. 2000) and neuropeptide metabolism (Sánchez et al. 2009a). They also induce behavioural modifications in the diploid *A. parthenogenetica* by increasing time spent near water surface and changing the phototaxis to positive (Sánchez et al. 2007), thus facilitating

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predation by birds and increasing the probability of the parasite transmission to final hosts (see e.g. Sánchez et al. 2009b). Cestodes were shown to infect native brine shrimp species (*A. parthenogenetica* and *A. salina*) at higher rates than the invasive *A. franciscana* and therefore may influence the outcome of their competitive interactions (Georgiev et al. 2007). Nearly all these studies were carried out on the basis of examination of adult (mature) specimens of brine shrimps, without considering the possible host potential of crustacean larvae and juveniles. The only exceptions are two studies paying attention to *Flamingolepis liguloides* infections in both adults and larval stages of *Artemia*, i.e. those by Gabrion et al. (1982) on the diploid *A. parthenogenetica* population from the saltmarshes of the Camargue (France) and by Mura (1995) on the bisexual *A. salina* population from a hypersaline pond at Su Pallosu (Sardinia, Italy).

The natural populations of *Artemia* spp. usually consist of individuals of various generations: mature reproducing individuals, juveniles, metanauplii and nauplii. Until now, scarce data have been published on the possible role of juveniles and metanauplii for the circulation of avian cestodes, though predation on these stages is similar to that on mature brine shrimps and they could also play a role in the parasite transmission. In addition, nothing is known about the effect of the cestode cysticercoids on larvae and juveniles of *Artemia*.

The aim of the present study is to examine the possible role of early developmental stages of *A. parthenogenetica* for the circulation of avian cestodes in natural conditions.

Material and methods

Sampling, processing and parasite identification

We examined a live sample of a diploid parthenogenetic *Artemia* population collected on 31 October 2007 from La Mata Lagoon (38°02'08"N, 0°42'30"W) located on the Mediterranean coast of the Province of Alicante, Spain. Surface temperature (15.5°C) and salinity (55 g.L⁻¹) in the brine were measured by portable equipment. The brine shrimps at that time in the lagoon were exclusively diploid parthenogenetic and no specimens of the autochthonous bisexual *Artemia salina* were recorded. Parthenogenetic populations of *Artemia* in the Mediterranean Region are currently designated under the binomen *A. parthenogenetica* Bowen and Sterling, 1978 (see Abatzopoulos et al. 2002), though there can be strains of various ploidy as previous morphological data unveiled by reporting also a tetraploid strain in the neighbouring salterns of Bonmatí (Amat et al. 1995).

Sampling was performed with a planktonic mesh hand net (160 µm mesh size) from a backwater where deep brine accumulation (110 g.L⁻¹) allowed to obtain a representative sample of the population. Collected specimens were transferred alive into 20 L plastic containers filled with lagoon brine and transported to the laboratory. In the laboratory, samples were kept into 60 L containers with 100 g.L⁻¹ brine and provided with gentle air bubbling from the bottom. Phytoplankton mass culture (*Dunaliella salina* plus *Tetraselmis suecica*) was added as food. The age structure of the population was examined by assessing the presence of various developmental stages. A sample of 2 L was sieved through a 1 mm mesh eye size. The brine shrimps were sorted by size and examined anaesthetized with chloroform under

stereomicroscope in order to record their age-group affiliation. There were 1968 specimens: 195 adults, 444 juveniles (immature), 1167 metanauplii and 162 nauplii. Later on, each individual was studied under a microscope for the presence of cestode larvae. The infected specimens were killed by heating to about 80°C and fixed in 70% ethanol for further identification of cysticercoids. Within several weeks, each specimen was prepared as a temporary glycerol whole-mount and the cysticercoids were identified under compound microscope after gentle pressing on the cover slip. If the identification of the cysticercoids was not possible at this stage, whole brine shrimps or isolated cysticercoids were prepared as permanent mounts in Berlese's medium to facilitate observations of the rostellar hooks. The identification of the cysticercoids was based on the characters described earlier (Georgiev et al. 2005; Vasileva et al. 2009), mostly using the shape, size and number of the rostellar hooks.

The identification of the age groups was performed after size measurements of anaesthetized individuals under stereomicroscope provided with a micrometer eyepiece. The differentiation between immature shrimps and castrated females was uncertain because immature juveniles and castrated mature females showed a similar aspect, i.e. an empty or underdeveloped ovisac but different size. In order to fix the minimum size to differentiate juveniles from mature castrated females, 27 ovigerous uninfected females were measured (mean length \pm SD, 10.14 \pm 0.70 mm) and 10 mm was selected as the differing size between the two groups. Adult females were classified in three groups according to their colour, i.e. (i) transparent, (ii) intensely bright red and (iii) light-red coloured, on the basis of previous observations on their infection levels (Gabrion et al. 1982; Amat et al. 1991b; Sánchez et al. 2006).

For the developmental stages of metacestodes, we used the terms: (i) individuals in process of scolex morphogenesis (further referred to as *mg*); (ii)

individuals with fully-formed scoleces and rostellar hooks but still not encysted, i.e. not invaginated into the cyst (further referred to as ‘individuals before invagination’, *bi*) and (iii) ripe cysticercoids, which are entirely formed and their scolex and neck are invaginated into the cyst (further referred to as ‘invaginated cysticercoids’, *ic*). These terms correspond to the gradual developmental stages of hymenolepidid cysticercoids as described by Skrjabin and Mathevossian (see Chervy 2002).

The definitions of the infection descriptors, i.e. prevalence (P), mean intensity (MI) and mean abundance (MA), correspond to those given by Bush et al. (1997).

Bird census data

Information about the presence of water birds in La Mata Lagoon during the period 2006-2007 was obtained from the monthly census performed by ornithologists in the Paraje Natural de las lagunas de Torrevieja y La Mata, Consellería de Medio Ambiente de la Generalitat Valenciana (government of the autonomous Valencian Community).

Statistical analysis

The quantitative descriptors of the infection (P, MI and MA) were calculated for the overall cestode infection and for each cestode species. To compare the cestode prevalence among the three age class groups (metanauplii, juveniles and adults), we used Chi-square tests. The differences between mean intensity and mean abundance were analysed with Kruskal-Wallis tests and multiple comparison procedure (Dunn’s method). P values were always Bonferroni-corrected to avoid type I errors (Miller

1991). In order to assess the effect of parasites on adult individuals, we used different statistical tests: Mann-Whitney U test was employed to compare cestode mean intensity between intensely bright red and light-red coloured individuals. The mean sizes of transparent, light-red and intensely-red specimens have been compared through one-way ANOVA followed by Tukey's test for multiple comparisons. Statistical analyses were carried out using SPSS 15.0 for Windows package (SPSS Inc., Chicago, IL, USA) and Sigma Plot 11.0 for Windows (Systat Software Inc., Germany).

Results

Cestode cysticercoids were found in three age classes: metanauplii (Figs. 1 and 2), juveniles and adults (Table 1). No cestodes were recorded in the nauplii examined. The prevalence of cysticercoids was significantly higher in adults (50.3%) than in early developmental stages: 9.5% and 11.8% in metanauplii and juveniles, respectively (Chi-square test, $P < 0.001$). The mean abundance was also significantly higher in adults (0.897) compared to both metanauplii (0.096) and juveniles (0.158) (Kruskal-Wallis test plus Dunn's method, $P < 0.05$). However, there were no significant differences in these two descriptors between metanauplii and juveniles. We recorded statistically significant differences in mean intensity between the three age groups (Kruskal-Wallis test, $P < 0.001$) exhibiting the highest value for the adults and the lowest one for the metanauplii. The levels of infection were lower in the earlier stages of *Artemia* and increased with the development of shrimps (Table 1).

Site of infection seemed to shift from thorax to abdomen with the increase of the body size. In metanauplii and juveniles, cysticercoids were predominantly located

in the thorax (94.8% and 85.7%, respectively). In adults, however, cysticercoids were most frequently found in the abdomen (Fig. 3).

Cysticercoids of four cestode species were identified in the sample studied: *Flamingolepis liguloides*, *F. flamingo*, *Confluaria podicipina* and *Eurycestus avoceti*. All they were recorded in adult brine shrimps. Only two of them (*F. liguloides* and *F. flamingo*) were found in metanauplii and juveniles (Table 1). These two cestode species showed statistically significant differences in prevalence in adults when compared to that in metanauplii plus juveniles (Chi-square test, $P < 0.05$). The mean abundance of *F. liguloides* was significantly higher in adults.

The length of infected juveniles ($N = 52$) was 7.50 ± 2.15 mm, ranging between 4 and 10 mm. The length of infected metanauplii ranged 1.94-4.53 mm (av. 3.20 ± 0.948 mm). Infection was recorded in all size groups of metanauplii, with no trend of increasing the intensity in the biggest metanauplii (Table 2). The cysticercoids of *F. liguloides* in metanauplii showed various degrees of development. There were cysticercoids at the stage of scolex morphogenesis, cysticercoids at the stage before invagination (Fig. 1) and fully formed cysticercoids (Fig. 2). In bigger metanauplii, the occurrence of fully-developed cysticercoids prevailed over the presence of developing cysticercoids (Fig. 4). All the recorded cysticercoids of *F. flamingo* were fully-developed, with one of them found in the size group of metanauplii 2.01-2.50 mm long.

Adult female brine shrimps were classified in three groups on the basis of their colour and reproductive status: (i) mature ovigerous (ovoviviparous) transparent individuals, (ii) mature ovigerous (ovoviviparous) light-red coloured individuals, and (iii) mature castrated intensely-red individuals. These groups showed differences relative to the cysticercoid infection (Table 3). The overall cysticercoid intensity

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3 ranged 1-6 in intensely-red individuals and 1-3 in light-red individuals. Within the
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5 two categories of coloured specimens, intensely-red and light-red, there were
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7 significant differences in cysticeroid mean intensity (Mann-Whitney U test, $P <$
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9 0.05). Significant differences were also found in the mean size between these three
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11 groups intensely-red, light-red and transparent shrimps (one-way ANOVA, $P < 0.05$).
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13 In the case of metanauplii and juveniles, both infected and uninfected specimens were
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15 transparent. Adult intensely-red castrated females ($N = 60$) were 11.60 ± 1.10 mm
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17 long. They showed the presence of cysticeroids of all the four cestode species
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19 recorded (Table 3). Mature light-red coloured females ($N = 38$) were 10.95 ± 0.87 mm
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21 long; they were infected with the same cestode species as the previous group (Table
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23 3). No infection was recorded in adult transparent individuals.
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32 **Discussion**

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34 This work deals with a brine shrimp population autochthonous for La Mata Lagoon
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36 and belonging to the diploid *A. parthenogenetica*. La Mata is a large hypersaline
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38 lagoon (700 Ha) used as a brine reservoir for the industrial cycle of the neighbouring
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40 salt pans at Torrevieja Lagoon (1,400 Ha). La Mata does not function in a similar way
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42 to evaporating ponds in sea solar salterns due to the diverse origin of its brine: rain
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44 water, sea water and high-salinity brine originating from diluted effluents from a salt
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46 mine. These summarised characteristics rule the hydrological and biological
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48 parameters of the ecosystem, sometimes showing fair biological productivity but
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50 usually functioning as an oligotrophic system (Amat et al. 1991a). This lagoon
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52 harbours brine shrimp populations of both bisexual *A. salina* and parthenogenetic
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54 diploid *A. parthenogenetica*, developing a turnover cycle along the year, with the
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56 bisexual species present during the colder seasons (winter and spring) and the
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parthenogenetic one occurring exclusively during the warm seasons, from the late spring to the end of the year (Amat 1981; Amat et al. 1995).

La Mata Lagoon is a protected area (Paraje Natural de las Lagunas de La Mata y Torrevieja; Ramsar site) where periodical waterfowl census is performed by park keepers. Seasonal census carried out in 2006, from January-February to the end of summer, showed the considerable abundance of grebes *Podiceps nigricollis* being 49% of species of aquatic birds that are potential final hosts of cestodes occurring in brine shrimps. Greater flamingos *Phoenicopterus ruber* were scarce in the lagoon, representing 0.30% of water birds in 2006, while waders (*Himantopus himantopus*, *Recurvirostra avosetta*, *Charadrius* spp., *Calidris* spp. and *Tringa* spp.) and gulls (*Larus ridibundus* and *L. genei*) represented 18.4% and 32.2%, respectively (Fig. 5). This composition of the aquatic bird community corresponds to the species identification of the recorded cysticercoids in brine shrimps. The definitive hosts of cestode parasites found as cysticercoids are grebes for *Confluaria podicipina*, flamingos for *Flamingolepis liguloides* and *F. flamingo*, and waders (and, to lesser extent, flamingos) for *Eurycestus avoceti*. For reviews of cestodes parasitising brine shrimps, see Georgiev et al. (2005) and Vasileva et al. (2009).

The structure of the examined brine shrimp population and the characteristics of its parasite infection had developed under the specific climatic conditions preceding our sampling. The area of La Mata lagoon suffered a drought from the end of 2006 to the end of the summer of 2007. Practically, there was no *Artemia* in the lagoon during spring – summer 2007 and water birds were scarce. The September (49.50 mm) – October (101.80 mm) rains alleviated the situation, lowering the salinity to 55 g.L⁻¹. These rains allowed mass hatching of *Artemia* cysts and the sampling carried out in October revealed a well-developed brine shrimp population, with

substantial proportion of juvenile and larval individuals. Grebes *P. nigricollis*, absent during September 2006 – July 2007, attained 870 individuals in October 2007. Monthly census performed during July – October 2007 (Fig. 6) showed a rapid increase of bird numbers, attaining in October values nearly tenfold higher than those registered during the previous three months. Among water birds associated with cestode parasites as potential definitive hosts, grebes attained 34.8%, flamingos 9.7%, waders 43.0% and gulls 12.5% (Fig. 5).

The present results demonstrate that metanauplii and juvenile brine shrimps can act as intermediate hosts in the life cycles of at least two cestode species, *Flamingolepis liguloides* and *F. flamingo*. In *Artemia* populations containing a substantial proportion of larval and immature individuals, their role for parasite circulation can be essential. Thus, in the population studied, only 36.3% of the cysticeroids of *F. liguloides* occur in adult brine shrimps; the remaining 63.7% are parasitic in metanauplii (39.6%) and juvenile individuals (24.1%). Similarly, the metacestodes of *F. flamingo* are also distributed among various age groups, i.e. in adults (44.4% of cysticeroids), juveniles (27.8%) and metanauplii (27.8%). The other two cestode species occurring in the studied *Artemia* population use adult brine shrimps only as intermediate hosts.

A possible explanation of the differences of the infections of metanauplii and juveniles with various cestode species can be based on the size of the parasite eggs. Cyclophyllidean tapeworm eggs, released into the brine with feces of birds, participate in the brine microplankton. In order to infest brine shrimps, they should be captured by the *Artemia* filtering system and swallowed. Robert and Gabrion (1991) emphasised the small dimensions of the eggs of *Flamingolepis liguloides* (30 × 45 µm), which are perfectly adapted as plankters with their numerous radial filaments on

the outermost egg envelope. The eggs of *Flamingolepis flamingo* have never been described. The eggs of *Eurycestus avoceti* are highly elongate, measuring about $110 \times 12 \mu\text{m}$, with two long polar filaments on their outer shell (Baer 1968). *Confluaria podicipina* has “large oval eggs”, measuring up to $63 \times 42 \mu\text{m}$ (Maksimova 1981). Recent studies on *Artemia franciscana* filtering behaviour (Gelabert 2001) reported variation in the size of particles (starch globules) swallowed by brine shrimps of various length. Thus, individuals (obviously metanauplii) up to 2 mm long were able to swallow particles with size $4\text{--}18 \mu\text{m}$, those with length 2–3 mm ingested particles of size $4\text{--}23 \mu\text{m}$, and those 3–4 mm and 4–5 mm long swallowed particles with size between 7 and $28\text{--}29 \mu\text{m}$ long. Dobbeleir et al. (1980) used glass microglobules with a diameter of up to $120 \mu\text{m}$, concluding that the maximum size of particles swallowed is $25\text{--}30 \mu\text{m}$ for nauplii and $40\text{--}50 \mu\text{m}$ for adult brine shrimps (*A. franciscana*). Similar studies on the feeding of nauplii, metanauplii and juveniles of *A. parthenogenetica* are lacking. However, the present results suggest that the relatively small eggs of *Flamingolepis* spp. might be ingested by preadult stages of the diploid parthenogenetic strain while the large eggs of *Eurycestus avoceti* and *Confluaria podicipina* can be swallowed only by adult brine shrimps.

Thus, only the two species occurring in flamingos (*Flamingolepis liguloides* and *F. flamingo*) infect metanauplii and juvenile brine shrimps in the population examined. According to Jenkin (1957), the usual food for flamingos with “shallow-keeled bills” as *Phoenicopterus ruber* are nearly exclusively some small aquatic invertebrates, most of them immature molluscs not exceeding 5 mm in size, small crustaceans and few other organisms. The food particles swallowed by flamingos range in size between $20\text{--}30 \mu\text{m}$ for some green algae and diatoms to 35 mm for oligochaetes or 20 mm for adult brine shrimps (Jenkin 1957). However, most of the

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3 food in natural conditions ranged between 0.50 and 4.50 mm, thus corresponding very
4 well to the size of *Artemia* nauplii and metanauplii. Therefore, these larval stages may
5 serve as efficient intermediate hosts for cestodes parasitising flamingos, which might
6 be an ecological interaction developed among coevolved predator, prey and their
7 common parasites (Peterson and Page 1988; Peterson et al. 1998; Fellowes and Travis
8 2000). In contrast, the black-necked grebes forage primarily on brine shrimps
9 (Caudell and Conover 2006; Sánchez et al. 2009b) by picking them from the surface
10 or while diving. This method of feeding is obviously associated with selecting
11 preferentially big adult and red shrimps, which are easy to be seen. It is likely that the
12 small-sized early stages of *Artemia* are not fit prey for grebes, so they should be
13 avoided as potential hosts for *Confluarina podicipina*. Similarly, Sánchez et al. (2009b)
14 demonstrated that sandpipers select red adult brine shrimps rich in both carotenoids
15 and parasites, thus making adult *Artemia* more suitable as an intermediate host for the
16 transmission of *Eurycestus avoceti* than larval stages of the same species. Though the
17 data presented are of limited value to be extrapolated to other cases of cestode
18 parasitism, it is safe to say that *Artemia* larval stages are suitable hosts for species
19 transmitted to a filter-feeding predator and do not play an efficient role for the life
20 cycles where the predator feeds by picking prey.
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46 There is no evidence of any special effect of cestode cysticeroids on the
47 development of *Artemia* metanauplii and juveniles. Cysticeroids of *F. liguloides* and
48 *F. flamingo* prefer the thorax of these premature hosts for their site of infection,
49 possibly because in this body part there is more space for the development of the cyst
50 and the cercomer. The only visible effect is a deformation that can produce an
51 accentuated bend in their aspect (Fig. 2).
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Notwithstanding, the penetration of *Flamingolepis* spp. oncosphere into *Artemia* individuals and their subsequent transformation into cysticeroids is possible during the whole life cycle of the brine shrimp. The presence of two or three *F. liguloides* cysticeroids in metanaupliar brine shrimps is extremely rare. In spite of this, it is possible to find much more numerous *F. liguloides* ripe cysticeroids in adult females. This is the case, e.g., in the diploid parthenogenetic *Artemia* population from the old solar saltern located at Calp (Province of Alicante) in the Spanish Mediterranean shore (38°38' N, 00°04' E). Adult females (average 11.13 mm long) sampled on 21 July 1999 in this saltern showed an intensity of 24 cysticeroids of *F. liguloides*. In this sample, we found a 17.26 mm long female having 63 cysticeroids. The presence of *F. liguloides* cysticeroids in this population allowed a viviparous reproduction attaining a mean fecundity of 36 embryos per brood, 50% as living nauplii and 50% as abortive embryos (F. Amat, unpublished data). These remarkable intensities of cysticeroids of *F. liguloides* did not produce relevant mortalities or suppress egg-laying.

In summary, the early developmental stages (metanauplii and juveniles) of *A. parthenogenetica* are efficient intermediate hosts in the life cycles of cestode parasites such as *F. liguloides* and *F. flamingo*, which occur in filter-feeding aquatic birds as flamingos. However, they do not play role for the life-cycle accomplishment of cestodes parasitising aquatic birds feeding on adult *Artemia* as usual preys.

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Figure captions

Fig. 1. A metanauplius of *Artemia* infected with one cysticercoid of *Flamingolepis liguloides* at the stage before invagination.

Fig. 2. A metanauplius of *Artemia* infected with one fully-developed cysticercoid of *Flamingolepis liguloides* in the thorax.

Fig. 3. Site of infection of cysticercoids (%) in each of *Artemia* age groups studied.

Fig. 4. Developmental stages of cysticercoids of *Flamingolepis liguloides* (%) in size groups of infected metanauplii. Abbreviations: *ic*, invaginated cysticercoids; *bi*, individuals before invagination; *mg*, individuals in process of scolex morphogenesis.

Fig. 5. Composition of water bird in La Mata Lagoon considering the bird species that are potential definitive hosts of cestodes parasitising brine shrimp (*Artemia parthenogenetica*) as intermediate host. The census data presented describe the situation during two consecutive years: winter-summer 2006 and summer-autumn 2007.

Fig. 6. Abundance of water birds in La Mata Lagoon according to the census performed during two consecutive years, 2006 and 2007

Table 1. Cestode infection in the age groups of *Artemia parthenogenetica* in the examined sample. The results of the examination of the nauplii (N = 162) are not shown (all individuals were uninfected). N, number of studied specimens; P, prevalence; MI, mean intensity \pm SE; MA, mean abundance \pm SE

	Metanauplii			Juveniles			Adults		
	N = 1167			N = 444			N = 195		
	P	MI	MA	P	MI	MA	P	MI	MA
Cestodes (total)	9.5	1.01 \pm 0.10	0.096 \pm 0.30	11.8	1.35 \pm 0.09	0.158 \pm 0.02	50.3	1.79 \pm 0.10	0.897 \pm 0.08
<i>Flamingolepis liguloides</i>	9.2	1.00 \pm 0.00	0.092 \pm 0.29	11.8	1.25 \pm 0.07	0.147 \pm 0.02	34.9	1.44 \pm 0.09	0.503 \pm 0.06
<i>Flamingolepis flamingo</i>	0.4	1.00 \pm 0.00	0.004 \pm 0.07	1.1	1.00 \pm 0.00	0.011 \pm 0.01	4.1	1.00 \pm 0.00	0.041 \pm 0.01
<i>Confluaria podicipina</i>	0.0	0.00	0.00	0.0	0.00	0.00	28.7	1.14 \pm 0.05	0.328 \pm 0.04
<i>Eurycestus avoceti</i>	0.0	0.00	0.00	0.0	0.00	0.00	2.6	1.00 \pm 0.00	0.026 \pm 0.01

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Table 2. Relative abundance (RA%) of the size groups among infected *Artemia metanauplii* and mean intensity (MI) of cysticercoid infection in each group

Size group	RA%	MI
≤ 2.00 mm	0.9	1.00
2.01-2.50 mm	4.6	1.00
2.51-3.00 mm	26.9	1.03
3.01-3.50 mm	23.2	1.00
3.51- 4.00 mm	12.0	1.00
> 4.00 mm	32.4	1.00

Table 3. Adult individuals of *Artemia parthenogenetica* in the examined sample, with reference to their infection status, colour appearance and reproductive status. N, number of specimens studied; Cyst. #, total number of cysticercoids recorded; *F.l.*, *Flamingolepis liguloides*; *F.f.*, *Flamingolepis flamingo*; *C.p.*, *Confluaria podicipina*; *E.a.*, *Eurycestus avoceti*; SE, standard error; SD, standard deviation

	N	Colour	Reproductive status	Length: mean \pm SD (in mm)	Infection status						
					Intensity		Cyst. #	Cestode species composition (%)			
					Mean \pm SE	Range		<i>F. l.</i>	<i>F.f.</i>	<i>C.p.</i>	<i>E.a.</i>
Infected	60	Intensely-red	Castrated	11.60 \pm 1.10	2.00 \pm 0.14	1-6	120	47.5	4.2	45.0	3.3
Infected	38	Light-red	Ovigerous	10.95 \pm 0.87	1.45 \pm 0.10	1-3	55	74.5	5.5	18.2	1.8
Non-infected	97	Transparent	Ovigerous	10.14 \pm 0.70							



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Fig. 2. A metanauplius of *Artemia* infected with one fully-developed cysticercoid of *Flamingolepis liguloides* in the thorax.

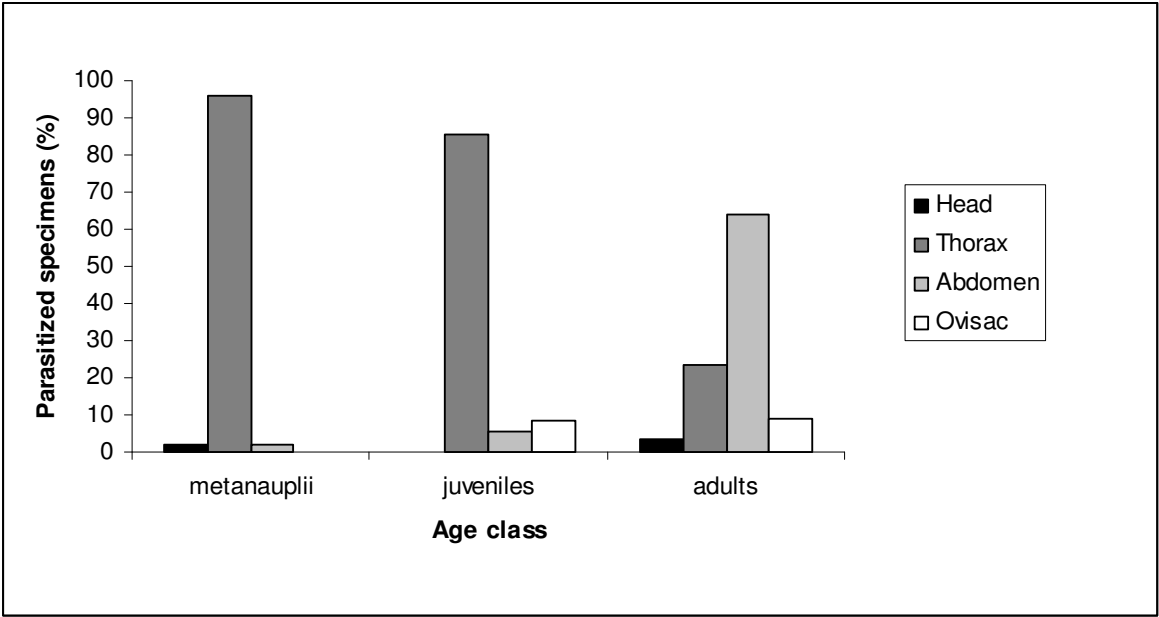


Fig. 3. Site of infection of cysticercoids (%) in each of *Artemia* age groups studied.

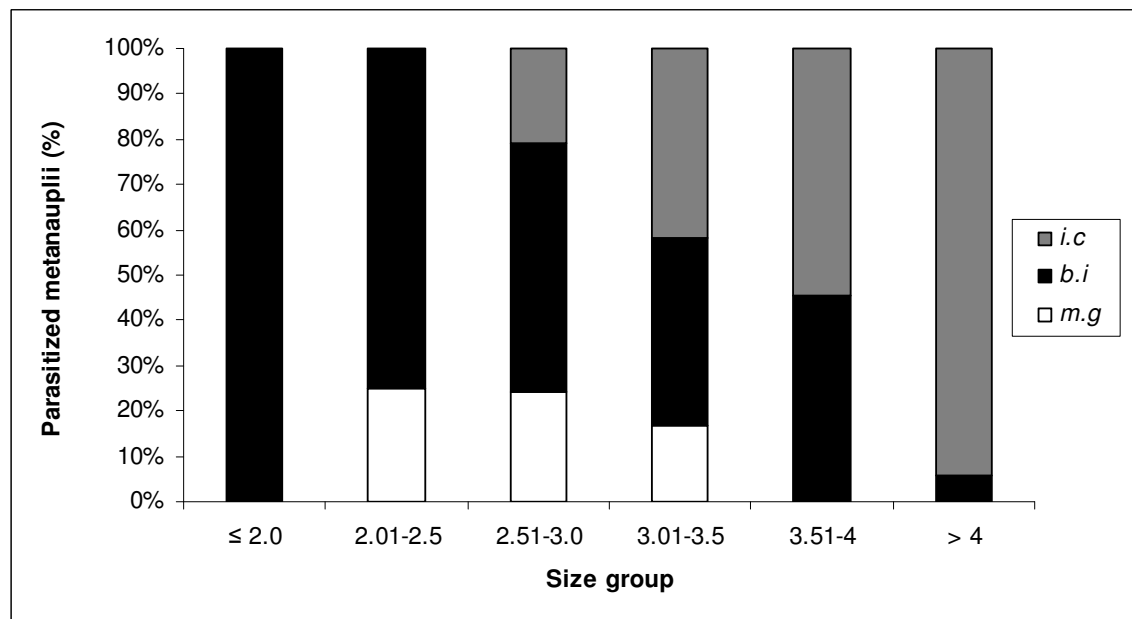


Fig. 4. Developmental stages of cysticercoids of *Flamingolepis liguloides* (%) in size groups of infected metanauplii. Abbreviations: *ic*, invaginated cysticercoids; *bi*, individuals before invagination; *mg*, individuals in process of scolex morphogenesis.

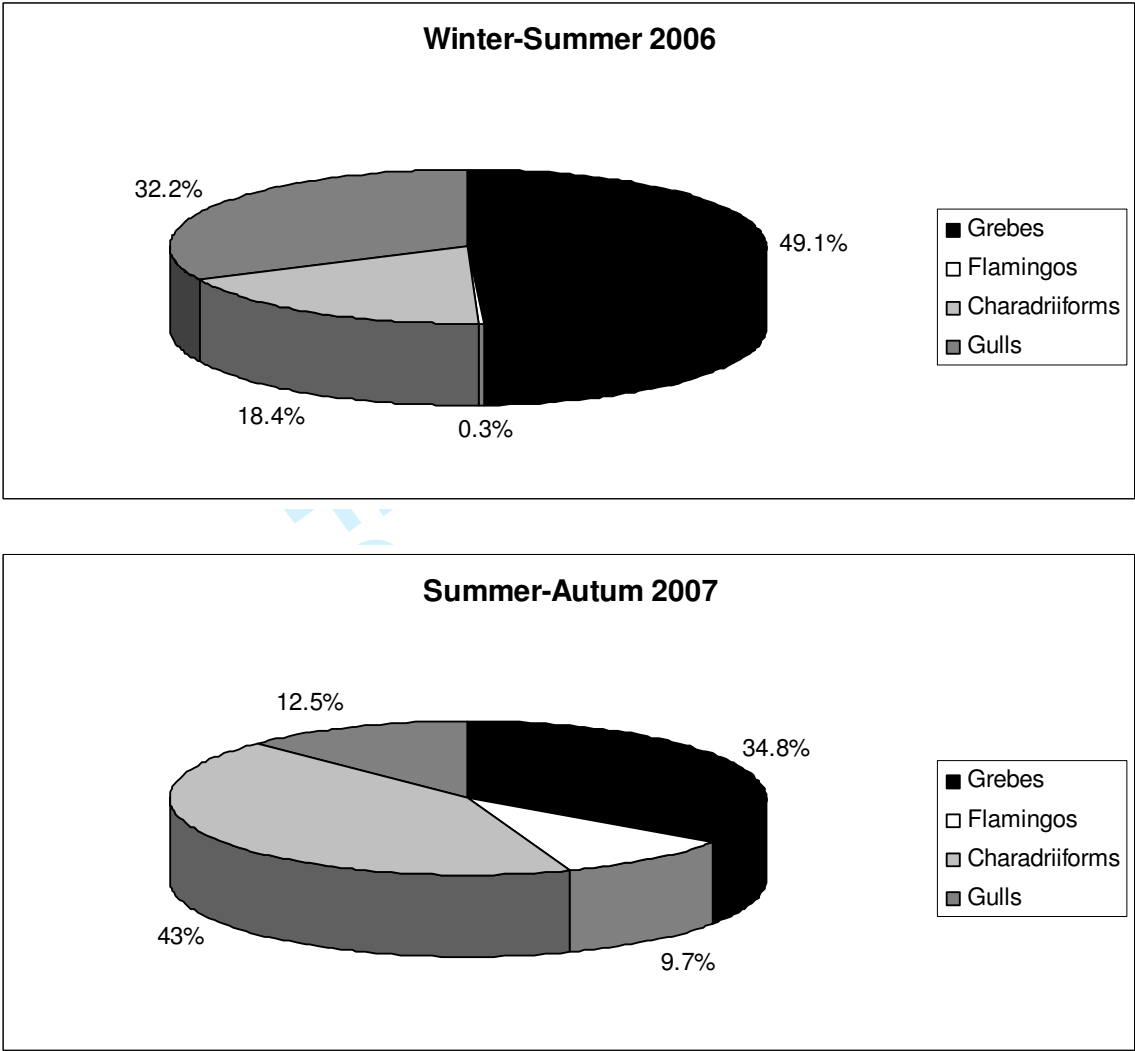


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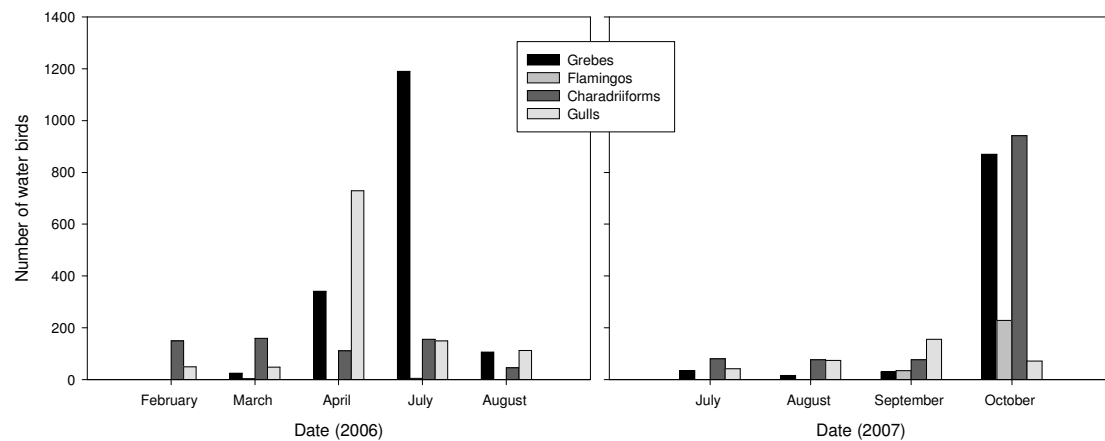


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