Comparing cestode infections and their consequences for host fitness in two sexual branchiopods: alien *Artemia franciscana* and native *A. salina* from mixed-populations.

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The American brine shrimp *Artemia franciscana* is invasive in the Mediterranean region where it has displaced native species (the sexual *A. salina*, and the clonal *A. parthenogenetica*) from many salt pond complexes. *Artemia* populations are parasitized by numerous avian cestodes whose effects have been studied in native species. We present a study from the Ebro Delta salterns (NE Spain), in a salt pond where both *A. franciscana* and native *A. salina* populations coexist, providing a unique opportunity to compare the parasite loads of the two sexual species in syntopy. The native species had consistently higher infection parameters, largely because the dominant cestode in *A. salina* adults and juveniles (*Flamingolepis liguloides*) was much rarer in *A. franciscana*. The most abundant cestodes in the alien species were *Eurycestus avoceti* (in adults) and *Flamingolepis flamingo* (in juveniles). The abundance of *E. avoceti* and *F. liguloides* was higher in the *A. franciscana* population syntopic with *A. salina* than in a monospecific population sampled at the same time in another pond where the native brine shrimp was absent, possibly because the native shrimp provides a better reservoir for parasite circulation. Infection by cestodes caused red colouration in adult and juvenile *A. salina*, and also led to castration in a high proportion of adult females. Both these effects were significantly stronger in the native host than in *A. franciscana* with the same parasite loads. However, for the first time, significant castration effects (for *E. avoceti* and *F. liguloides*) and colour change (for six cestode species) were observed in infected *A. franciscana*. Avian cestodes are likely to help *A. franciscana* outcompete native species. At the same time, they are likely to reduce the production of *A. franciscana* cysts in areas where they are harvested commercially.

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Introduction

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28 The American brine shrimp *Artemia franciscana* (Kellog, 1906) is a key species for the aquaculture

29 industry and a model organism for laboratory research (e.g. in toxicology, genetics or physiology). *A.*

30 *franciscana* cysts, particularly originating from Great Salt Lake (USA), have been exported worldwide

31 for aquaculture, the improvement of salt extraction in salt ponds and the pet trade market, facilitating

32 the arrival and spread of *A. franciscana* outside its natural range (Amat et al., 2005; Ruebhart, Cock &

33 Shaw, 2008; Vikas et al., 2012). In the Western Mediterranean, the introduction of this exotic species is

34 provoking the extinction of native *A. salina* (Leach, 1819) and *A. parthenogenetica* (Bowen & Sterling,

35 1978) populations (Amat et al., 2005; Amat et al., 2007; Muñoz et al., 2014). The ability of *A.*

36 *franciscana* to outcompete other *Artemia* species rapidly in the field may be largely explained by a

37 higher reproductive rate that often allows it to eliminate native congeners within a few generations in

38 the laboratory (Browne, 1980; Browne & Halanych, 1989; Amat et al., 2007). However, parasites can

39 also influence biological invasions, depending on their relative impacts on native and alien species

40 (Hatcher, Dick & Dunn, 2006; Prenter et al., 2004; Dunn et al., 2012).

41   *Artemia* spp. are intermediate hosts of avian cestodes that can have a major influence on their

42 fitness. In the Mediterranean, native brine shrimps are parasitized by 12 species of avian tapeworms

43 whose final hosts are flamingos, waders, grebes, ducks or gulls (Georgiev et al., 2005; Georgiev et al.,

44 2007; Vasileva et al., 2009). To elucidate the role of parasites in an invasion it is important to study

45 parasite infections in populations of native and alien host species co-ocurring in the same habitat

46 (syntopic populations), but this is difficult because native *Artemia* have already disappeared from most

47 sites where *A. franciscana* is detected. Comparisons of allopatric *Artemia* populations from the

48 southern Iberian Peninsula suggest that *A. franciscana* populations have lower levels of infections by

49 cestodes than the native sexual *A. salina* and the clonal *A. parthenogenetica* (Georgiev et al., 2007;

50   Georgiev et al., 2014; Sánchez et al., 2013). In the present study, we take advantage of a unique

51   opportunity to compare the parasitism of *A. franciscana* and *A. salina* in the only site where these two

52   sexual species are known to coexist: the Ebro Delta salterns in north-east Spain.

53   Sánchez et al. (2012) compared parasitism in syntopic host populations of *A. franciscana* and *A.*

54   *parthenogenetica* in southern France, and found lower cestode diversity and abundance in the invasive

55   host. This could potentially be because the sexual invasive species can resist parasites better than the

56   clonal native one, owing to the importance of genetic recombination in resisting parasites (Red Queen

57   hypothesis: Van Valen, 1973; Moritz et al., 1991; Mee & Rowe, 2006). However, in the case of two

58   sexual species, the relative effects of parasites may be more similar. Given the lack of preexisting data,

59   comparing parasite impacts in two bisexual hosts (*A. franciscana* and *A. salina*) is of considerable

60   interest and importance in the context of the biological invasion.

61   Trophically transmitted parasites such as larval helminths with complex life cycles often induce

62   changes in the physiology, behaviour or appearance of intermediate hosts ("host manipulation"),

63   rendering them more vulnerable to predation and facilitating transmission to final hosts (Barber, Hoare

64   & Krause, 2000; Moore, 2002; Poulin, 2006). In native Mediterranean *Artemia* populations, cestode

65   infections are associated with a reddish colour, positive phototaxis, gigantism, longer life span, reduced

66   fecundity and higher levels of lipids and glycogen (Thiéry, Robert & Gabrion, 1990; Amat et al., 1991;

67   Robert & Gabrion, 1991; Sánchez et al., 2006; Sánchez, Georgiev & Green, 2007; Amarouayache,

68   Derbal & Kara, 2009; Sánchez et al., 2009a). The pathogenic impact of endemic cestodes on the alien

69   *A. franciscana* remains unclear, particularly when it co-occurs with native congeners. If *A. franciscana*

70   is less affected by cestodes, this may help explain its invasion success and help it to outcompete native

71   hosts.

72   In the present study, we compared the cestode infections between *A. franciscana* and *A. salina*

73   in a salt pond where they coexisted. We tested the hypothesis that *A. franciscana* is less susceptible to

74 endemic cestodes than *A. salina*, leading to a competitive advantage that contributes to the invasion

75 success. Secondly, we compared the infections between this *A. franciscana* population that is

76 coexisting with a native host (*A. franciscana*-syntopic population), and a neighbouring pond where

77 there are no congeners (*A. franciscana*-monospecific population), in order to explore if there is a

78 negative relationship between community diversity and disease risk (Schmidt & Ostfeld, 2001;

79 Keesing, Holt & Ostfeld, 2006; Johnson &Thieltges, 2010). We predict that parasite loads of *A.*

80 *franciscana* would be lower when coexisting with *A. salina*, because many infective cestode eggs

81 would be removed by the native host. Thirdly, we analyzed the consequences of infection by different

82 cestode species for colour change and reproductive activity in both *Artemia* species. We predict fewer

83 consequences for the alien host, owing to weaker host-parasite coevolution.

84

85 **Materials and methods**

86 *Study system and field samples*

87 The Ebro Delta (Province of Tarragona, NE Spain) is the largest wetland area (320 km^2) in the

88 Mediterranean coast of Spain and is protected as a Natural Park, Ramsar site and an EU Special

89 Protection Area for birds. *Artemia franciscana* was first detected in our study area (*La Trinitat* coastal

90 salterns, 40º35´N, 00º41´E, Fig. 1) in 2007 (Amat et al., 2007). Previously, these salterns supported a

91 tetraploid parthenogenetic population of *Artemia* (Amat et al., 1995), but this native taxon has not been

92 recorded since.

93 Repeated sampling visits were carried out from 2007 to 2010 inclusive. The present study

94 focuses on samples collected at the following two ponds during a 12 month period from January 2009

95 to January 2010, when the native *A. salina* was detected in coexistence with *A. franciscana*. 1) Pond

96 CX, a large pond situated between other salt ponds and the sea, isolated from the brine circulation

97 system (Fig. 1), was the only pond in which *A. franciscana* coexisted with *A. salina*. Samples were

98   collected monthly from January 2009 to January 2010 (salinity S range = 45 - 260 g/L, mean ± s.e. =

99   114.6 ± 18.35; temperature T range = 5 - 32ºC, mean ± s.e. = 18.1± 2.37). 2) Pond 4 was sampled

100 monthly from January to March 2009, then in January 2010 (S range = 125 - 150 g/L, mean ± s.e. =

101 132.5 ± 5.95; T range = 8.5 – 12ºC, mean ± s.e. = 10.9 ± 0.83). In order to increase the sample size for

102 infected shrimps, additional samples of *A. franciscana* collected on other dates and in other ponds (S.

103 Redón, AJ Green, BB Georgiev, GP Vasileva, F Amat, 2015 unpublished) were used when considering

104 the influence of cestodes on colouration and reproductive activity of this host species.

105   *Artemia* samples were collected from each pond with 160 μm and 500 μm mesh hand-nets and

106 transported alive to the laboratory. Living *Artemia* individuals, anaesthetized with a few drops of

107 chloroform saturated distilled water, were examined under a stereomicroscope and juveniles and adults

108 were separated. Juveniles are immature specimens with sexual segments (ovisac or hemipenis) that are

109 not completely developed. Juveniles and adults were identified to species after Amat (1985); Hontoria

110 & Amat (1992a) and Hontoria & Amat (1992b). The proportion of juveniles varied over time (Fig. S1)

111 confirming that both species had multiple generations per year (Amat et al., 2007). We differentiated

112 juveniles and adults by sex (Amat, 1985). Adult females with empty ovisac and no signs of functional

113 ovaries were considered to be castrated and those with embryos, naupliae, or cysts filling the ovisac, or

114 ovulating (oocites moving along the ovaries or filling the oviducts) were considered as ovigerous (Fig.

115 S3). The colour of adults and juveniles was assigned to three categories: dark-red, light-red and not-red

116 (Fig. S2). This research was conducted under permit from the Ebro Delta Natural Park office provided

117 to FA.

118 *Parasite identification*

119 Juvenile and adults were examined, while lightly anaesthetized, under a stereomicroscope for cestode

120 cysticercoids, until a total of approximately 400 individual shrimps (when available) was reached,

121 including juveniles and adults. After observations of the cysticercoids *in situ*, each infected specimen

122   was prepared in a temporary glycerol mount and examined under a compound microscope.

123   Identification was based on Georgiev et al. (2005) and Vasileva et al. (2009). More details of sampling

124   protocols are provided by S Redón, AJ Green, BB Georgiev, GP Vasileva, F Amat, 2015 unpublished.

125   *Quantitative analysis of cestode infection and statistics*

126   Several descriptors were applied to the cestode infections in *Artemia*. Prevalence (P%: proportion of

127   infected individuals in the host population), abundance (MA: mean number of cysticercoids for the

128   total number of shrimps examined) and intensity (MI: mean number of cysticercoids in infected

129   shrimps) were calculated for the overall infection and for each parasite species (terminology following

130   Bush et al., 1997). Also, we quantified species richness (SR: mean number of cestode species present

131   in each *Artemia* individual) and the species richness in infected specimens (SRinfected).

132   *i) Host species: invader vs. native*

133   To compare cestode infections between host species, we used monthly samples from pond CX in

134   which a total of 2150 shrimps were examined (1252 *A. franciscana* and 898 *A. salina*). On a month by

135   month basis, Mann-Whitney *U* tests were employed to compare the abundance of parasites, the

136   infection intensity and SR between host species, separately for adults and juveniles. Differences in the

137   prevalence of cestodes between host species were analyzed with Fisher Exact tests.

138   *ii) Influence of coexistence with A. salina on parasitism in A. franciscana*

139   We compared the parasite infections in *A. franciscana* in a mixed species populations (presence of *A.*

140   *salina*, pond CX) with those in a single species populations sampled simultaneously (pond 4). Using

141   Fisher Exact and Mann-Whitney *U* tests, we compared the cestode infections in *A. franciscana*

142   between ponds on the same sampling day during three months (January, February and March 2009),

143   separately for adults and juveniles.

144   *Parasite castration effects*

145 Taking advantage of all *A. franciscana* samples available (including those from other dates and ponds;

146 S. Redón, AJ Green, BB Georgiev, GP Vasileva, F Amat, 2015 unpublished), we compared the

147 proportion of infected and uninfected females that were castrated. The castration effect of cestodes in

148 *A. franciscana* was evaluated by Wilcoxon tests for paired samples, comparing the proportions of

149 castrated shrimps within a given sample, thus avoiding any non-independence of observations of

150 different individuals within the same sample. In contrast, Fisher Exact tests were applied for *A. salina*,

151 because the small number of samples with this species obliged us to pool them and treat each

152 individual shrimp as an independent observation. Since castration was never recorded in uninfected

153 female *A. salina*, this pooling was unlikely to bias the observed levels of castration.

154 *Effects of parasites on colour*

155 In order to assess the effects of parasites on the colour of *Artemia* individuals, we compared the

156 proportion of red individuals (summing the "dark-red" and "light-red" categories, Fig. S2) between

157 infected and non-infected specimens with Fisher Exact tests. The same test was employed to analyze

158 colour-effects between host sexes and host species for both adults and juveniles. In addition, to test the

159 influence of infection intensity on colour*,* Mann-Whitney *U* tests were applied to compare the cestode

160 intensity between infected individuals that were red and those that were not. All statistical analyses

161 were carried out using SPSS 15.0 for Windows package (SPSS Inc., Chicago, IL, USA).

162 **Results**

163 *Comparing parasite loads in A. franciscana and A. salina in a syntopic population*

164 In pond CX where both *Artemia* species coexisted, *A. salina* was detected only in colder months, from

165 January to May 2009, and September 2009 to January 2010 (Fig. 2), whereas *A. franciscana* was

166 present all year long. Nine cestode species were detected in *A. franciscana*, and seven of these in *A.*

167 *salina* (Table 1). These included four species whose adults are parasitic in flamingos (*Flamingolepis*

168 *liguloides*, *F. flamingo*, *Gynandrotaenia stammeri* and *Gynandrotaenia* sp.), three in shorebirds

169   (*Anomotaenia tringae*, *A. microphallos* and *Eurycestus avoceti*), one in gulls (*Wardium stellorae*) and

170   one in shelducks (*Fimbriarioides tadornae*).

171   <u>Adult brine shrimps</u>

172   A total of 868 adults were examined in samples with both species present. Of these, 24.9% of *A. salina*

173   and 19.1% of *A. franciscana* were parasitized by cysticercoids (Table 1). Cestodes were detected in all

174   five months of co-existence (Fig. 3). For overall infection, *A. salina* had a higher prevalence than *A.*

175   *franciscana* (Table 1), with statistically significant differences in April and May (Fig. 3A).

176   Mean total cestode abundance varied from 0.02 to 1.35 in *A. salina* and from 0.06 to 0.42 in *A.*

177   *franciscana*, and was significantly higher in *A. salina* in April and May (Mann-Whitney *U* tests, $p <$

178   0.05). Total cestode intensity ranged from 1 to 1.84 in *A. salina* and 1 to 1.19 in *A. franciscana*, and

179   was significantly higher in the former in May ($p < 0.001$). The relative abundance of cestode species

180   varied between hosts. *F. liguloides* was the most prevalent and abundant parasite in *A. salina* (Table 1),

181   and its prevalence and abundance were significantly lower in *A. franciscana* in March, April and May

182   (Fig. 3B). In May, the prevalence and abundance of *A. tringae* were also significantly higher in *A.*

183   *salina* (41.2% and 0.471 ± 0.08, respectively) than *A. franciscana* (11.6% and 0.126 ± 0.03). Except in

184   January, *E. avoceti* was the most prevalent and abundant parasite in *A. franciscana* (Table 1). No

185   significant differences in the intensity of individual cestode species were recorded (Table 1).

186   *A. salina* had a higher SR of cestodes (Table 1), with significantly higher SR in April (0.70 ±

187   0.15 for *A. salina* *vs*. 0.13 ± 0.09 for *A. franciscana*, $p < 0.05$) and May (1.12 ± 0.11 *vs*. 0.40 ± 0.04, *p*

188   < 0.001). In May, the SR of infected specimens was also significantly higher for *A. salina* (1.52 ± 0.09

189   *vs*. 1.12 ± 0.04, $p < 0.001$).

190   <u>Juvenile brine shrimps</u>

191   A total of 1282 juveniles were examined in samples with both species present. Of these, 9.5% of *A.*

192   *salina* and 3.9% of *A. franciscana* were parasitized by cysticercoids (Table 1). Cestodes were detected

193 in all months of co-existence except January (Fig. 4). For the overall infection, prevalence and

194 abundance were significantly higher in *A. salina* from March to May (Fig. 4A). No significant

195 differences were detected in intensity between host species (Table 1).

196 　　　　Prevalence and abundance of *F. liguloides* were significantly higher in *A. salina* from March to

197 May (Fig. 4B). Prevalence and abundance of *A. tringae* were also significantly higher in *A. salina* in

198 May (66.7% *vs*. 5.7%, and 0.778 ± 0.22 *vs*. 0.086 ± 0.06). SR was significantly higher for *A. salina* in

199 March (0.18 *vs*. 0.04; *p* = 0.001), April (0.05 *vs*. 0.00; *p* < 0.001) and May (1.56 *vs*. 0.63; *p* = 0.003).

200 However, no significant differences were detected for SR of infected specimens.

201 *The influence of coexistence with native A. salina on parasitism in A. franciscana*

202 For adult *A. franciscana*, higher total prevalence and abundance of cestodes were recorded in each of

203 three months in pond CX where it was coexisting with *A. salina* (AF-mixed) than in pond 4 where it

204 was the only species present (AF-single), with statistically significant differences in March (Table 2).

205 Prevalence was significantly higher in AF-mixed for *F. liguloides* and *E. avoceti* in January and

206 March, respectively (Table 2). SR was significantly higher in AF-mixed in March (Table 2). No

207 significant differences were detected for infection intensity.

208 　　　　Amongst juvenile *A. franciscana*, total prevalence, abundance and species richness were

209 significantly higher in AF-mixed in March (as for adults), but significantly lower in AF-mixed in

210 January (when no infected juveniles of either *Artemia* species were recorded in the mixed population).

211 The abundance of *F. flamingo* in March was also significantly higher for AF-mixed (Table 3).

212

213 *Influence of parasites on host colouration*

214 Red colouration was strongly associated with presence of cestodes in both *Artemia* species, and in both

215 adults and juveniles (Fig. 5). Red colouration was only observed in uninfected shrimps in the case of

216 two of 8260 uninfected adult *A. franciscana*. In infected adults, red colouration was much more

217   frequent in *A. salina* (26.8%) than *A. franciscana* (5.3%, Fisher Exact test, $p < 0.001$). There were no

218   differences between sexes for either host species in the probability of redness when infected.

219   For both *Artemia* species, there was a positive relationship between infection level (in terms of

220   species richness and/or intensity of infection) and the likelihood of red colour.

221   For infected adults, the total number of cysticercoids was higher in red individuals both for *A.*

222   *franciscana* (mean ±s.e. = 2.4 ±0.18 when red, 1.23 ± 0.02 when not; Mann-Whitney *U* test, $U =$

223   11280, $p < 0.001$) and *A. salina* (2.08 ± 0.20 when red, 1.31 ± 0.07 when not; $U = 487$, $p < 0.001$).

224   Owing to the larger sample size, the positive correlation between infection intensity and probability of

225   red colouration was particularly clear in *A. franciscana* adults (Fig. 6). The same pattern was apparent

226   for the dominant cestode species, *E. avoceti* (results not shown).

227   For *A. franciscana* adults infected by a single cysticercoid, four cestode species significantly

228   increased the chance of red colouration compared to uninfected shrimps: *E. avoceti*, *A. microphallos*,

229   *A. tringae* and *G. stammeri* (Fisher Exact test, $p < 0.001$). A significant increase in redness was also

230   detected for *Flamingolepis* when intensity (I) was higher than one (I = 2 for *F. liguloides* and I = 3 for

231   *F. flamingo*).

232   For *A. franciscana* juveniles infected by a single cysticercoid, three cestode species

233   significantly increased the chance of red colouration compared to uninfected shrimps: *F. flamingo*, *A.*

234   *microphallos*, and *E. avoceti* (Fisher Exact test, $p < 0.006$). When pooling simple infections of different

235   intensities, a significant redness effect was also detected for *F. flamingo* (Fisher Exact test, $p < 0.001$).

236   For *A. salina* adults infected by a single cysticercoid cestode species, *A. tringae* ($p < 0.001$) and

237   *E. avoceti* ($p < 0.024$) significantly increased the chance of red colouration compared to uninfected

238   shrimps. No colour-effects were detected for individuals infected by *F. liguloides* or the other cestode

239   species, independently of the intensity of the infection.

240   Simple infection by a single *A. tringae* cysticercoid was significantly more likely to cause

241   redness in adult *A. salina* (N = 10, of which 70% were red) than in *A. franciscana* (N = 21, with 9.5%

242   red) (Fisher Exact test, *p* = 0.001). No significant differences between host species in colour-effects

243   were detected for other cestode species.

244

245   *Cestodes and host castration*

246   In *A. franciscana* adults, 11.2% of infected females (N = 553) were castrated (i.e. with empty ovisac

247   and no signs of functional ovaries, Fig. S3). Amongst non-infected females (N = 3267), a significantly

248   lower proportion (6.1%) were castrated (Wilcoxon Signed Rank test, 35 paired samples, *T* = 7, *p* =

249   0.001, Fig. 6).

250   Among infected, castrated female *A. franciscana*, 42 (68%) were infected by only one parasite

251   species (i.e. single infections), and of these most were infected with *E. avoceti* (64.3%) or *F. liguloides*

252   (19%). Castration rates in females infected only by *E. avoceti* were only slightly but significantly

253   higher (7.5%) than those in uninfected females (Wilcoxon Signed Rank test, 30 paired samples, *T* = 7,

254   *p* = 0.019). A similar effect was observed for *F. liguloides* (Fisher Exact Test, *p* = 0.001), for which

255   25% of infected females (N = 32) were castrated. For *E. avoceti*, the probability of castration clearly

256   increased with the intensity of infection, and 35.7% of females with ≥3 cysticercoids were castrated (*p*

257   = 0.001). Unlike *E. avoceti*, infection with a single *F. liguloides* cysticercoid had a significant

258   castration effect (p = 0.004). No significant castration effect was detected for the remaining cestode

259   species, but sample sizes were low. Of female *A. franciscana* infected by more than one cestode

260   species (co-infections) (N = 57), 35.1% were castrated. Among these castrated females (N = 20), *E.*

261   *avoceti* was present in 18 individuals and *F. flamingo* in seven, with six other cestode species present

262   in smaller numbers.

263   For *A. salina*, castration was recorded in 22.5% of infected females (N = 40) but no uninfected

264   females (Fisher Exact Test, *p* < 0.001, Fig. 7). Among the nine castrated, infected females, eight

265   (88.9%) were infected by *F. liguloides* (exclusively or in co-infections with *A. tringae* or *E. avoceti*)

266   and the other was infected by a single *A. tringae*. If we consider females with small broods (<10 eggs

267   in the ovisac) and absence of oocytes migrating in the ovaries, the proportion of female *A. salina*

268   infected with *F. liguloides* with limited fecundity increases markedly from 35.7% to 57.1%.

269

270   **Discussion**

271   We present a unique study comparing the cestode infections and their consequences for two closely

272   related branchiopod crustaceans. The native *A. salina* generally had more cestodes than the alien *A.*

273   *franciscana*. When coexisting with the native, the alien had higher rates of infection than when present

274   in a monospecific population. Although the cestodes were shown to have important effects on the

275   fecundity and colour of both host species, these effects were stronger in the native specie.

276

277   <u>Comparing infections in syntopic *A. franciscana* and *A. salina:* how important is co-evolution?</u>

278   Comparative studies of parasite infections in native and alien host species are essential to understand

279   the role of parasites in biological invasions (Dunn, 2009; Kelly et al., 2009; Dunn et al., 2012). The

280   present work sheds light on the role of endemic cestodes in competitive interactions between native

281   and alien sexual *Artemia*. Cestode parasitism differed clearly between invasive and native brine

282   shrimps under the same environmental conditions and at the same time. Cestode species richness and

283   the prevalence of several species were lower in *A. franciscana*, and the difference was especially

284   striking for *F. liguloides* (Figs. 3, 4). These results are consistent with studies of monospecific

285   populations in the southern Iberian Peninsula which have found *A. franciscana* to have low infection

286   rates throughout the annual cycle. In summer, Georgiev et al. (2007) found lower levels of cestode

287 infections in three *A. franciscana* populations than in an allopatric *A. salina* population. Studies

288 throughout the annual cycle of *A. franciscana* in Cadiz Bay and of *A. salina* in Almería also found that

289 the invasive species had fewer infections (Sánchez et al., 2013; Georgiev et al., 2014). Our results for

290 syntopic populations indicate that these earlier results are not explained by other differences between

291 these allopatric populations (e.g. in environmental conditions, or in the density of birds). A previous

292 study in syntopic populations in Aigües-Mortes in France showed that *A. franciscana* had fewer

293 cestodes than native, clonal *A. parthenogenetica* (Sánchez et al., 2012), although this might be related

294 to the advantages of sex in resisting parasites. In any case, the present study provides strong evidence

295 that the introduced *A. franciscana* is a less susceptible host for cestodes than the native sexual *A.*

296 *salina*.

297   The lower burden of parasites in introduced hosts suggests *A. franciscana* is resistant to several

298 endemic cestode species such as *F. liguloides*. Different capacities to infect the new host may be the

299 result of varying host-parasite co-evolutionary history. The lower infection of *F. liguloides* in *A.*

300 *franciscana* may be explained by a strong immune response of the host against this parasite, given the

301 restricted distribution of flamingos in North America which barely overlaps with the natural range of

302 *A. franciscana* (Muñoz et al., 2013). There are no flamingos in the western USA, from where *A.*

303 *franciscana* has been exported around the world. Observations of dead cysticercoids of *F. liguloides* in

304 *A. franciscana* support a strong immune response in this host (Georgiev et al., 2014). In contrast, the

305 high prevalence of *E. avoceti* recorded in *A. franciscana* in our study may reflect a shared

306 coevolutionary history, since *E. avoceti* occurs in shorebirds in North America (Clark, 1954). *A.*

307 *franciscana* does not appear to be especially resistant to cestodes in its native range, since the total

308 prevalence of cestodes in Great Salt Lake, USA was much higher than in the Ebro Delta and other parts

309 of the invasive range (Redón et al., in press). Our results are consistent with previous studies that have

310 found parasites of native congeners ("endemic parasites") to be unable to spread to invaders or able to

311   infest a newly introduced host to a lesser extent (Dunn & Dick, 1998; Torchin, Byers & Todd, 2005;

312   Genner, Michel & Tood, 2008).

313

314   <u>How does coexistence of both species influence infection parameters in *Artemia franciscana*?</u>

315   When a non-indigenous species arrives in a new range, it can affect native communities through

316   different processes: "spillover" of introduced parasites, "spillback" of native parasites, and "dilution"

317   of parasitism (Kelly et al., 2009). *A. franciscana* was introduced into Europe as imported cysts

318   (dormant eggs) free of native cestode parasites, so "spillover" is not expected in this system. The low

319   levels of parasitism recorded in *A. franciscana* indicate that it is a poor or incompetent reservoir for the

320   endemic cestodes, making "spillback" to native *Artemia* unlikely. We might expect a parasite dilution

321   effect of the invader to the benefit of the native host which should be subjected to lower disease

322   transmission when mixed with incompetent, alien hosts (Keesing, Holt & Ostfeld, 2006; Hall et al.,

323   2009; Johnson & Thieltges, 2010). A reduced infection rate by native trematodes was demonstrated

324   experimentally in native freshwater snails *Potamopyrgus antipodarum* exposed together with the

325   invader *Lymnaea stagnalis* (Kopp & Jokela, 2007). Given the strong effects of cestodes on *A. salina*,

326   such a "dilution effect" could increase the possibility of local persistence of *A. salina* in the presence of

327   the invader (Hatcher, Dick & Dunn, 2006).

328   Because our study area did not include ponds holding only *A. salina*, we cannot reliably assess

329   the strength of any dilution effect on this species. However, the levels of cestode prevalence we

330   recorded in the presence of *A. franciscana* were not unusually low compared to studies of *A. salina*

331   elsewhere in the absence of the invader (Table S1). Likewise, Sánchez et al. (2012) found no evidence

332   of a dilution effect on *A. parthenogenetica* when coexisting with *A. franciscana*, since the total

333   prevalence in *A. parthenogenetica* was high (c.70%).

334   Our study allowed a good test of the "dilution effect" in the other direction, i.e. whether the

335   native host reduced parasitism in the alien host. We found the opposite effect (an "amplification

336   effect"), with higher infection in *A. franciscana* when coexisting with *A. salina*. Indeed, the temporal

337   dynamics of infection in *A. franciscana* in pond CX (with both *Artemia* species) seem to depend on the

338   proportion of native hosts in the *Artemia* population. Thus, total prevalence increased from January to

339   May 2009 (coinciding with the abundance of *A. salina*), then declined from June to December when *A.*

340   *franciscana* was the dominant species (Fig. S4). In contrast, in pond 4 (without *A. salina*), higher

341   prevalences were recorded in July and August during 2007 and 2008 (S Redón, AJ Green, BB

342   Georgiev, GP Vasileva, F Amat, 2015 unpublished data). Since *A. franciscana* is a poor reservoir (i.e.

343   the cestodes may not circulate effectively in them), adding *A. salina* to the community might increase

344   the prevalence of the infection in the alien host because the added host is a better reservoir (Keesing,

345   Holt & Ostfeld, 2006, Hatcher, Dick & Dunn, 2006). Since *Artemia* are only intermediate hosts, such

346   an explanation would require final avian hosts to be relatively faithful to individual ponds, so that birds

347   that become infected in the pond with *A. salina* are more likely to release cestode eggs there than in

348   other ponds. Another possible and not mutually exclusive explanation for our results is that the final

349   avian hosts are more attracted to ponds holding *A. salina* because this is their preferred prey, and this

350   leads to greater parasite circulation in these ponds. Owing to weak manipulation by native parasites, *A.*

351   *franciscana* is much less abundant at the water surface where they are accessible to birds, and as shown

352   in our study, they are less likely to have the red colour preferred by birds (Sánchez et al., 2009b). In

353   turn, this suggests that the generally low infection rates in *A. franciscana* may be accounted for not

354   only by a greater resistance to parasites than native species, but also by a tendency for final hosts

355   (birds) to avoid invaded salt ponds, reducing levels of parasite circulation. A study of how waterbird

356   use differs between salt ponds with native or alien shrimp is required.

357

358   <u>Castration and colour effects in native and alien hosts</u>

359   In many trophically transmitted parasites, larval parasite stages alter host characteristics such as

360   longevity, size, colour or behaviour to increase the risk of predation by the final host (Moore, 2002;

361   Thomas, Adamo & Moore, 2005; Médoc, Bollache & Beisel, 2006). Parasitic castrators benefit by

362   diverting host resources away from reproduction, allowing faster growth and earlier arrival at the

363   infective stage, or an increase in host lifespan, thus increasing opportunities for transmission (Hurd,

364   Warr & Polwart, 2001). *A. parthenogenetica* infected with *F. liguloides* live longer than uninfected

365   ones (Amat et al., 1991), and this increased host longevity may ensure the complete development of the

366   infective stage from oncosphere to ripe cysticercoid (see Redón et al., 2011), ready to be transmitted to

367   final hosts.

368   We found significant but mild castrating effects of cestodes in *A. franciscana* and much

369   stronger effects in native *A. salina*. Owing to the smaller sample size, for *A. salina* we could only

370   confirm a castrating effect for *F. liguloides* which was already known to have a strong castrating effect

371   on this species (Amarouayache, Derbal & Kara, 2009) and *A. parthenogenetica* (Amat et al., 1991;

372   Sánchez et al., 2012). In *A. franciscana*, we found a significant castration effect for *F. liguloides* and *E.*

373   *avoceti*, with a stronger effect in the former (a single cysticercoid of *F. liguloides* is able to castrate,

374   whereas two or more are needed for *E. avoceti*). This is the first time that a castration effect has been

375   demonstrated for dilepidid cestodes in *Artemia*, and suggests these effects are widespread and not

376   limited to *F. liguloides*. The number of adult females infected with other cestode species was too small

377   to detect mild castration effects. Our results have important implications for the use of *A. franciscana*

378   in aquaculture, since they show that cestode infections reduce host fecundity, even in the introduced

379   range. This suggests that cyst production in sites within the introduced range where cysts are harvested

380   for aquaculture (e.g. Bohai Bay in China) is likely to be reduced by cestode infections.

381   This is the first study of the colour-effects of cestodes in *A. franciscana* in the invasive range,

382   and the first to consider the effects in *A. salina* in detail. The association of red colouration with

383   cestode infection is well known for native *A. parthenogenetica*, especially for *F. liguloides*, and

384   appears to be due to parasite manipulation and the preference that final hosts have for predating red

385   *Artemia* (Sánchez et al., 2009b; Sánchez et al., 2012). However, it is also possible that this altered

386   pigmentation is caused by a host immune response against infection (e.g. given the antioxidant

387   function and immunostimulating properties of carotenoids) rather than a specific parasitic strategy to

388   ensure transmission. The red colour of infected *A. parthenogenetica* is partly due to carotenoids (Amat

389   et al., 1991; Sánchez et al., 2006) and carotenoids seem to play an important role in immune defence

390   by providing free radical scavengers against cestode infection (van der Veen, 2005). Cornet, Biard &

391   Moret (2007) found a positive relationship between carotenoid concentration in haemolyymph and

392   immune parameters (those evolved in the prophenoloxidase cascade) in the crustacean *Gammarus*

393   *pulex*, suggesting that carotenoids can help to reduce the costs of immunity by limiting self-harming. A

394   positive relationship between carotenoid concentration and the abundance of cestodes (but not of

395   nematodes) was also observed in greylag geese *Anser anser* (Figuerola et al., 2005), suggesting there

396   may be a similar host response to cestode infection across a broad taxonomic range.

397   The proportion of infected shrimps with a red colour was higher for *A. salina* than for *A.*

398   *franciscana*, whether considering co-infections or only single infections. This further suggests that the

399   invasive species has greater resistance to cestode infections. Red colouration in *A. salina* was

400   significantly associated with infections by *A. tringae* and *E. avoceti*, and the redness effect of *A.*

401   *tringae* was stronger than in *A. franciscana*. Surprisingly, we could not detect a significant effect of *F.*

402   *liguloides* on *A. salina* colour, suggesting that this native sexual host is more resistent to this parasite

403   than the asexual *A. parthenogenetica*, as predicted by the Red Queen hypothesis. However, red

404   colouration may be of less value to increase transmission to the filter-feeding flamingos that are final

405 hosts of *F. liguloides*, than to shorebirds which are visual predators and final hosts of *E. avoceti* and

406 *Anomotaenia* spp.

407   Red pigmentation in *A. franciscana* is linked with cestode infection, particularly with dilepidids

408 (*E. avoceti*, *A. tringae*, and *A. microphallos*), and to a lesser extent with flamingo parasites (*F.*

409 *liguloides* and *F. flamingo*), a finding in line with the higher prevalence and castration effects we

410 recorded for dilepidids. There was a strong positive correlation between red-colour and overall

411 infection-intensity, as previously observed for *A. parthenogenetica* (Sánchez et al. 2006). Ours is the

412 first study to compare redness effects separately in juvenile and adult *Artemia*. For *A. franciscana*

413 juveniles, we found a significant redness effect for *F. flamingo*, *E. avoceti* and *A. microphallos*,

414 suggesting that cestodes manipulate host colour even in early stages of host development. There is a

415 need for detailed research into the nature and function of the pigments causing red colouration in

416 different *Artemia* species and life stages parasitized by different cestodes.

417   As in the present study, previous studies in other host-parasite systems have found endemic

418 parasites to have less pathological effects in alien hosts than in native hosts (Bauer et al., 2000; Cornet,

419 Sorci & Moret, 2010). For example, in an acantocephalan-gammarid system, Cornet, Sorci & Moret

420 (2010) found that a local parasite *Pomphorhynchus laevis* had less ability to infect and induce

421 pathogenic effects in the invasive than in native gammarids.

422

423 *Consequences of the invasion of A. franciscana for native parasites*

424 Our results demonstrate that *A. franciscana* has accumulated novel parasites following its introduction

425 (see also S. Redón, AJ Green, BB Georgiev, GP Vasileva, F Amat, 2015 unpublished). However, it is

426 not clear that all the cestodes parasitizing native *Artemia* in the Mediterranean region will be able to

427 persist if all the native populations are replaced by the American species. Coextinctions (defined as the

428 loss of one especies as a result of the extinction of the species it depends on; Dunn et al., 2009) seem

429   likely in our host-parasite system, especially at a local scale of individual ponds. Cysticercoids have to

430   be able to establish, survive and grow until reaching an infective-stage in brine shrimps, and then reach

431   avian final hosts to complete the parasite´s cycle. It is possible that some cestodes will not be able to

432   infect this new host at a high enough rate or to reach their final hosts with a sufficiently high frequency

433   (according to the very low prevalences and the weaker manipulation observed) to ensure the survival of

434   a viable metapopulation. When introduced species become hosts for endemic parasites it does not

435   necessarily mean that they play a major role in the life cycles and transmission of these parasites. The

436   reduced levels of infection and reduced castration effects in *A. franciscana* suggest that cestodes may

437   not be able to infect or manipulate the alien host to a sufficient extent to ensure viability. The reduced

438   redness suggests that cestodes are less likely to reach final hosts (through bird predation) when

439   infecting alien *Artemia*, and in turn that the value of salterns for waterbird conservation may be

440   reduced by the invasion. Parasites are an important component of food webs (Lafferty, Dobson &

441   Kuris, 2006, Lafferty et al., 2008) and ecosystem functioning (Hudson, Dobson & Lafferty, 2006), so

442   loss of cestodes may have an impact on the stability of hypersaline ecosystems.

443

444   ***Conclusions***

445   Cestodes have a greater impact on native *A. salina* than on invasive *A. franciscana* in several ways,

446   suggesting that native cestodes are poorly adapted to these novel hosts. They cause infections of higher

447   prevalence and species richness in the native host. For a given infection intensity, they cause a greater

448   impact on host fecundity, and a greater change in colouration, likely to translate into a higher predation

449   rate by birds. Therefore, cestodes can influence competition between *Artemia* species and are likely to

450   help *A. franciscana* to outcompete native species in the Mediterranean region and other parts of the

451   world. In turn, the viability of these cestode populations across broad scales, where waterbird

452 populations interact with different hypersaline ecosystems, is threatened by the loss of native *Artemia*

453 which may act as a "source" for cestodes whereas *A. franciscana* populations may act as a "sink".

454

459

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663 invasion of the exotic *Artemia franciscana* and displacement of the autochthonous *Artemia* populations
664 from the hypersaline habitats of India. *Hydrobiologia* 684:129–142.

665

666 **Figure Captions**
667

668 Fig. 1. Geographical situation of the studied area. Location of the Ebro delta (Province of Tarragona,
669 NE Spain) and map of the Ebro Delta salterns *La Trinitat* indicating the *Artemia* collection sites: (1)
670 Pond 4, (2) Pond CX.

671

672 Fig. 2. Seasonal variation of temperature and proportion of native *A. salina* in the brine shrimp
673 community from pond CX throughout an annual cycle. The right vertical axis indicates the proportion
674 of all *Artemia* present (whether adults or juveniles) that were *A. salina*. *A. franciscana* were present in
675 all months.

676

677 Fig. 3. Comparative infection in adults of syntopic brine shrimp populations: *A. franciscana* and *A.*
678 *salina* from pond CX, during the co-existence period. (A) Total cestode prevalence, (B) Prevalence of
679 *Flamingolepis liguloides*. Bars show upper 95% confidence intervals. * significant differences at $p <$
680 0.05 according to Fisher Exact tests.

681

682 Fig. 4. Comparative infection in juveniles of syntopic brine shrimp populations: *A. franciscana* and *A.*
683 *salina* from pond CX, during the co-existence period. (A) Total cestode prevalence, (B) Prevalence of
684 *Flamingolepis liguloides*. Bars show upper 95% confidence intervals. * significant differences at $p <$
685 0.05 according to Fisher Exact tests. No cestodes were recorded in January.

686

687 Fig. 5. Proportion of infected adults and juveniles with red colouration for *A. salina* (AS) and *A.*
688 *franciscana* (AF). Bars show 95% confidence intervals. The total number of infected specimens in
689 each group is shown within the columns. Red colouration was not recorded in uninfected *A. salina*, and
690 was very rare in *A. franciscana* (absent in juveniles, 0.02% in adults). For all four categories the
691 proportion of red shrimps is significantly higher in infected individuals (Fisher exact, $p < 0.001$).

692

693　Fig. 6.　Colour pattern in relation to intensity of infection in adult *A. franciscana*. Bars show 95%

694　confidence intervals. The total number of infected specimens in each group is indicated.

695

696　Fig. 7.　Castration effects in adult female *A. franciscana* and *A. salina*. Bars show 95% confidence

697　intervals.

Table 1(on next page)

Comparative cestode infection in syntopic brine shrimp populations from pond CX (adults and juveniles).

P%, prevalence; *MI*, mean intensity; *MA*, mean abundance; ±SE, standard error. *SR* infected is the species richness of infected individuals. Only data for samples in which both *Artemia* species were recorded (N = 5) are presented.

Table 1. Comparative cestode infection in syntopic brine shrimp populations from pond CX (adults and juveniles). *P%*, prevalence; *MI*, mean intensity; *MA*, mean abundance; ±SE, standard error. *SR* infected is the species richness of infected individuals. Only data for samples in which both *Artemia* species were recorded (N = 5) are presented.

		Host - adults		Host - juveniles	
Cestode species		*A. franciscana* *N*=487	*A. salina* *N*=381	*A. franciscana* *N*=765	*A. salina* *N*=517
Flamingolepis liguloides (FL)	P%	0.8	17.6	1.4	8.3
	MI	1.00 ±0.00	1.25 ±0.07	1.18 ±0.18	1.21 ±0.11
	MA	0.008 ±0.004	0.221 ±0.03	0.017 ±0.01	0.101 ±0.02
Flamingolepis flamingo (FF)	P%	2.3	1.3	2.2	1.2
	MI	1.09 ±0.09	1.20 ±0.20	1.00 ±0.00	1.17 ±0.17
	MA	0.025 ±0.01	0.016 ±0.01	0.022 ±0.01	0.014 ±0.01
Wardium stellorae (WS)	P%	1.4	1.0	0.0	0.0
	MI	1.14 ±0.14	1.00 ±0.00	0.00	0.00
	MA	0.016 ±0.01	0.011 ±0.01	0.00	0.00
Fimbriarioides tadornae (FT)	P%	1.4	0.0	0.4	0.4
	MI	1.00 ±0.00	0.00	1.00 ±0.00	1.00 ±0.00
	MA	0.014 ±0.01	0.00	0.004 ±0.002	0.004 ±0.003
Eurycestus avoceti (EA)	P%	8.6	3.9	0.7	0.2
	MI	1.02 ±0.02	1.00 ±0.00	1.00 ±0.00	1.00
	MA	0.088 ±0.01	0.039 ±0.01	0.007 ±0.003	0.002 ±0.002
Anomotaenia tringae (AT)	P%	4.9	7.3	0.3	1.2
	MI	1.08 ±0.08	1.14 ±0.09	1.50 ±0.50	1.17 ±0.17
	MA	0.053 ±0.01	0.084 ±0.02	0.004 ±0.003	0.014 ±0.01
Anomotaenia microphallos (AM)	P%	0.8	0.0	0.0	0.0
	MI	1.00 ±0.00	0.00	0.00	0.00
	MA	0.008 ±0.00	0.00	0.00	0.00
Gynandrotaenia stammeri (GS)	P%	0.2	0.00	0.0	0.0
	MI	1.00	0.00	0.00	0.00
	MA	0.002 ±0.002	0.00	0.00	0.00
Gynandrotaenia sp. (GSP)	P%	0.4	0.8	0.1	0.0
	MI	1.00 ±0.00	1.00 ±0.00	1.00	0.00
	MA	0.004 ±0.003	0.008 ±0.005	0.001 ±0.001	0.00
Total infection	P%	19.1	24.9	3.9	9.5
	MI	1.15 ±0.04	1.52 ±0.08	1.40 ±0.13	1.41 ±0.14
	MA	0.220 ±0.02	0.378 ±0.04	0.055 ±0.01	0.134 ±0.02
Species Richness ±SE		0.21 ±0.02	0.32 ±0.03	0.05 ±0.01	0.11 ±0.02
SR infected		1.10 ±0.03	1.28 ±0.06	1.30 ±0.09	1.18 ±0.06

Table 2(on next page)

Comparative cestode infection in adult *A. franciscana* from single and mixed populations.

Parasite infections from a monospecific population (pond 4, AF-single) and one in co-existence with *A. salina* (pond CX, AF-mixed) from January to March 2009. The proportion of adult *Artemia* that were *A. franciscana* is shown in parentheses. *N*, total number of specimens examined; *S*, salinity. Other abbreviations are explained in Table 1. Fisher exact tests were used to compare infection rates between ponds in a given month. Significantly higher values are shown in bold and as $*p < 0.05$, $**p < 0.001$. --, test not possible owing to lack of data.

2 Table 2. Comparative cestode infection in adult *A. franciscana* from single and mixed populations. Parasite infections from a monospecific
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4 adult *Artemia* that were *A. franciscana* is shown in parentheses. *N*, total number of specimens examined; *S,* salinity. Other abbreviations are
5 explained in Table 1. Fisher exact tests were used to compare infection rates between ponds in a given month. Significantly higher values are
6 shown in bold and as *$p < 0.05$, **$p < 0.001$. --, test not possible owing to lack of data.

Cestode species		January 2009			February 2009			March 2009		
		AF-single N=963, S=125g/l	AF-mixed (75.9%) N=132, S=56g/l	p value	AF-single N=244, S=130g/l	AF-mixed (32.9%) N=28, S=65g/l	p value	AF-single N=186, S=150g/l	AF-mixed (33.8%) N=104, S=70g/l	p value
FL	P%	0.3	**3.0***	0.005	0.8	0.0	1.000	0.0	0.0	--
	MI	1.00 ±0.00	1.00 ±0.00	1.000	1.00 ±0.00	0.00	--	0.00	0.00	--
	MA	0.0031 ±0.00	**0.0303 ±0.01****	0.000	0.0082 ±0.01	0.00	0.631	0.00	0.00	1.000
FF	P%	0.7	2.3	0.011	2.5	0.0	1.000	0.0	1.0	0.359
	MI	1.14 ±0.14	1.00 ±0.00	0.833	1.00 ±0.00	0.00	--	0.00	1.00	--
	MA	0.0083 ±0.00	0.0303 ±0.01	0.081	0.0246 ±0.01	0.00	0.402	0.00	0.0096 ±0.01	0.181
WS	P%	0.0	0.0	--	0.0	0.0	--	0.5	0.0	1.000
	MI	0.00	0.00	--	0.00	0.00	--	1.00	0.00	--
	MA	0.00	0.00	1.000	0.00	0.00	1.000	0.0054 ±0.01	0.00	0.455
FT	P%	0.2	0.0	1.000	0.8	3.6	0.279	0.0	0.0	--
	MI	1.00 ±0.00	0.00	--	1.00 ±0.00	1.00	1.000	0.00	0.00	--
	MA	0.0021 ±0.00	0.00	0.600	0.0082 ±0.01	0.0357±0.04	0.187	0.00	0.00	1.000
EA	P%	1.7	0.8	0.710	0.8	3.6	0.279	0.0	**4.8***	0.006
	MI	1.00 ±0.00	1.00	1.000	1.00 ±0.00	1.00	1.000	0.00	1.00 ±0.00	--
	MA	0.0166 ±0.00	0.0076 ±0.01	0.431	0.0082 ±0.01	0.0357 ±0.04	0.187	0.00	**0.0481 ±0.02***	0.003
GS	P%	0.3	0.0	1.000	0.4	0.0	1.000	0.0	0.0	--
	MI	1.00	0.00	--	1.00 ±0.00	0.00	--	0.00	0.00	--
	MA	0.0041 ±0.00	0.00	0.521	0.0031 ±0.00	0.00	0.735	000	0.00	1.000
GSP	P%	0.5	0.0	1.000	0.0	0.0	--	0.0	1.9	0.128
	MI	1.00 ±0.00	0.00	--	0.00	0.00	--	0.00	1.00 ±0.00	--
	MA	0.0052 ±0.00	0.00	0.407	0.00	0.00	1.000	0.00	0.0192 ±0.01	0.058
Total infection										
	P%	3.7	6.1	0.232	5.3	7.1	0.658	0.5	**7.7***	0.001
	MI	1.03 ±0.03	1.00 ±0.00	0.917	1.00 ±0.00	1.00 ±0.00	1.000	1.00	1.00 ±0.00	1.000
	MA	0.0384 ±0.01	0.0606 ±0.02	0.204	0.0533±0.01	0.0714 ±0.05	0.691	0.0054 ±0.01	**0.0865 ±0.03***	0.001
Species Richness		0.04 ±0.01	0.06 ±0.02	0.203	0.05 ±0.01	0.07 ±0.05	0.691	0.01 ±0.01	**0.08 ±0.03***	0.001
SR infected		1.00 ±0.00	1.00 ±0.00	1.000	1.00 ±0.00	1.00 ±0.00	1.000	1.00	1.00 ±0.00	1.000

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Table 3 (on next page)

Comparative cestode infection in juvenile *A. franciscana* from single and mixed populations.

Parasite infections from a monospecific population (pond 4, AF-single) and one in co-existence with *A. salina* (pond CX, AF-mixed) from January to March 2009. Proportion of juvenile *Artemia* that were *A. franciscana* is shown in parentheses. *N*, total number of specimens examined. Other abbreviations are explained in Table 1. Fisher exact tests were used to compare infection rates between ponds in a given month. Significantly higher values are shown in bold and as *$p < 0.05$, **$p < 0.001$. --, test not possible owing to lack of data.

2 Table 3. Comparative cestode infection in juvenile *A. franciscana* from single and mixed populations. Parasite infections from a
3 monospecific population (pond 4, AF-single) and one in co-existence with *A. salina* (pond CX, AF-mixed) from January to March 2009.
4 Proportion of juvenile *Artemia* that were *A. franciscana* is shown in parentheses. *N*, total number of specimens examined. Other
5 abbreviations are explained in Table 1. Fisher exact tests were used to compare infection rates between ponds in a given month.
6 Significantly higher values are shown in bold and as *$p < 0.05$, **$p < 0.001$. --, test not possible owing to lack of data.
7

		January 2009			February 2009			March 2009		
Cestode species		*AF-single* N =168	*AF-mixed (65.7%)* N =134	*p* value	*AF-single* N =67	*AF-mixed (50.4%)* N =179	*p* value	*AF-single* N =248	*AF-mixed (59.4%)* N =111	*p* value
FL	P%	1.2	0.0	0.505	0.0	5.0	0.119	0.0	0.9	0.309
	MI	1.00 ±0.00	0.00	--	0.00	1.00 ±0.00	--	0.00	1.00	--
	MA	0.0119 ±0.01	0.00	0.206	0.00	0.0503 ±0.02	0.062	0.00	0.0090 ±0.01	0.135
FF	P%	1.8	0.0	0.257	1.5	1.7	1.000	0.0	1.8	0.095
	MI	1.33 ±0.33	0.00	--	1.00	1.00 ±0.00	1.000	0.00	1.00 ±0.00	--
	MA	0.0238 ±0.01	0.00	0.121	0.0149 ±0.01	0.0168 ±0.01	0.919	0.00	**0.0181 ±0.01***	0.034
EA	P%	1.8	0.0	0.257	0.0	0.0	--	0	0.9	0.309
	MI	1.00 ±0.00	0.00	--	0.00	0.00	--	0.00	1.00	--
	MA	0.0179 ±0.01	0.00	0.121	0.00	0.00	1.000	0.00	0.0090±0.01	0.135
GS	P%	0.6	0.0	1.000	0.0	0.0	--	0.0	0.0	--
	MI	1.00	0.00	--	0.00	0.00	--	0.00	0.00	--
	MA	0.0060 ±0.01	0.00	0.372	0.00	0.00	1.00	000	0.00	1.000
GSP	P%	0.6	0.0	1.000	0.0	0.6	1.00	0.0	0.0	--
	MI	1.00	0.00	--	0.00	1.00	--	0.00	0.00	--
	MA	0.0060 ±0.01	0.00	0.372	0.00	0.0056 ±0.01	0.541	0.00	0.00	1.000
Total infection										
	P%	**6.0***	0.0	0.003	1.5	5.6	0.298	0.0	**2.7***	0.029
	MI	1.10 ±0.10	0.00	--	1.00	1.30 ±0.15	0.727	0.00	1.33 ±0.33	--
	MA	**0.0655 ±0.02***	0.00	0.004	0.0149 ±0.01	0.0726 ±0.02	0.165	0.00	**0.0360 ±0.02***	0.009
Species Richness		**0.06 ±0.02***	0.00	0.004	0.01 ±0.01	0.07 ±0.02	0.165	0.00	**0.04 ±0.02***	0.009
SR infected		1.00 ±0.00		--	1.00	1.30 ±0.15			1.33 ±0.36	--

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Geographical situation of the studied area.

Location of the Ebro delta (Province of Tarragona, NE Spain) and map of the Ebro Delta salterns *La Trinitat* indicating the *Artemia* collection sites: (1) Pond 4, (2) Pond CX.

River Ebro Delta

Mediterranean sea

12.4 km

Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image © 2015 Institut Cartogràfic de Catalunya

La Trinitat salterns

1

2

827 m

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Seasonal variation of temperature and proportion of native *A. salina* in the brine shrimp community from pond CX throughout an annual cycle.

The right vertical axis indicates the proportion of all *Artemia* present (whether adults or juveniles) that were *A. salina*. *A. franciscana* were present in all months.
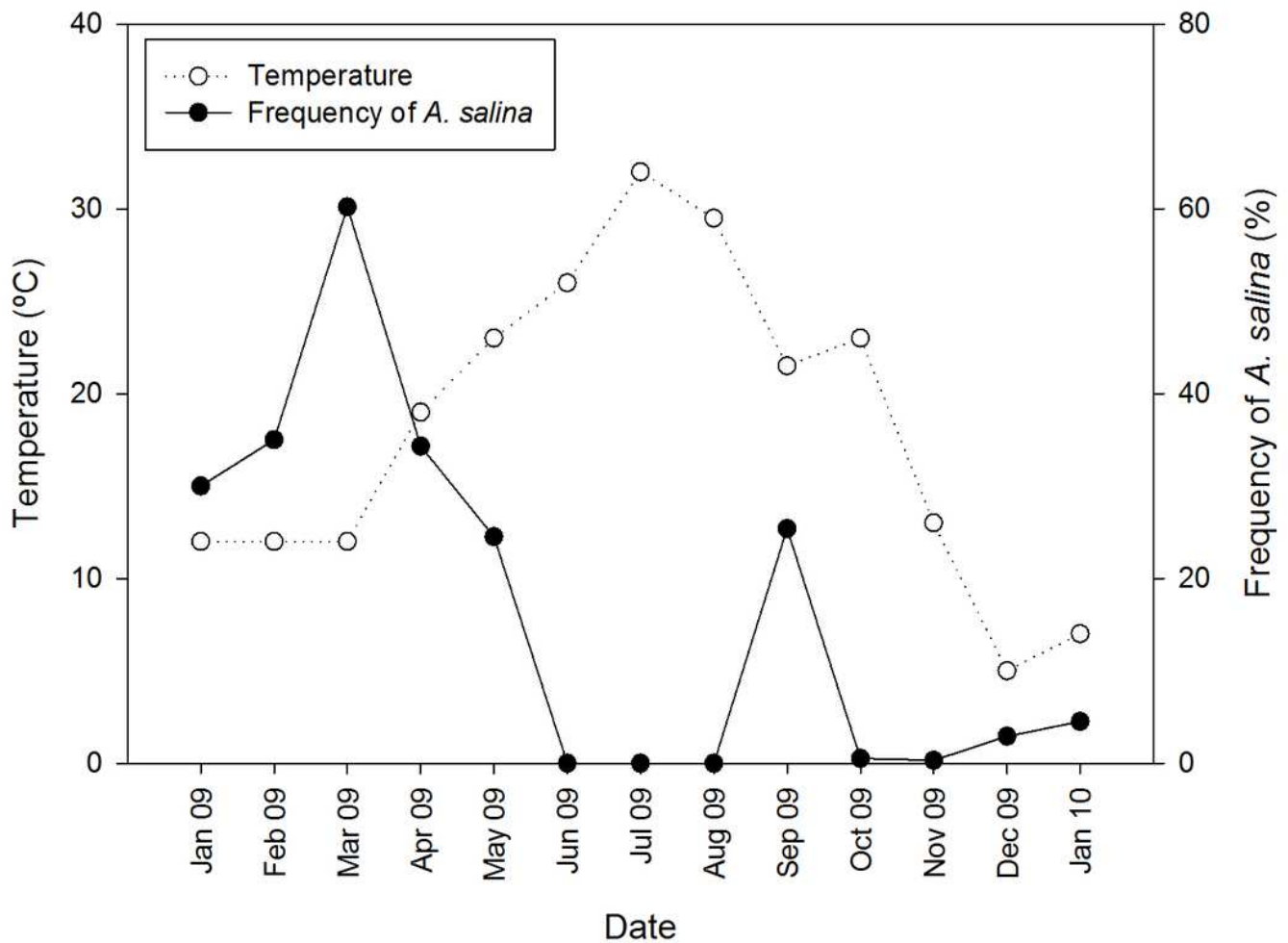
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Comparative infection in adults of syntopic brine shrimp populations: *A. franciscana* and *A. salina* from pond CX, during the co-existence period.

(A) Total cestode prevalence, (B) Prevalence of *Flamingolepis liguloides*. Bars show upper 95% confidence intervals. * significant differences at $p < 0.05$ according to Fisher Exact tests.

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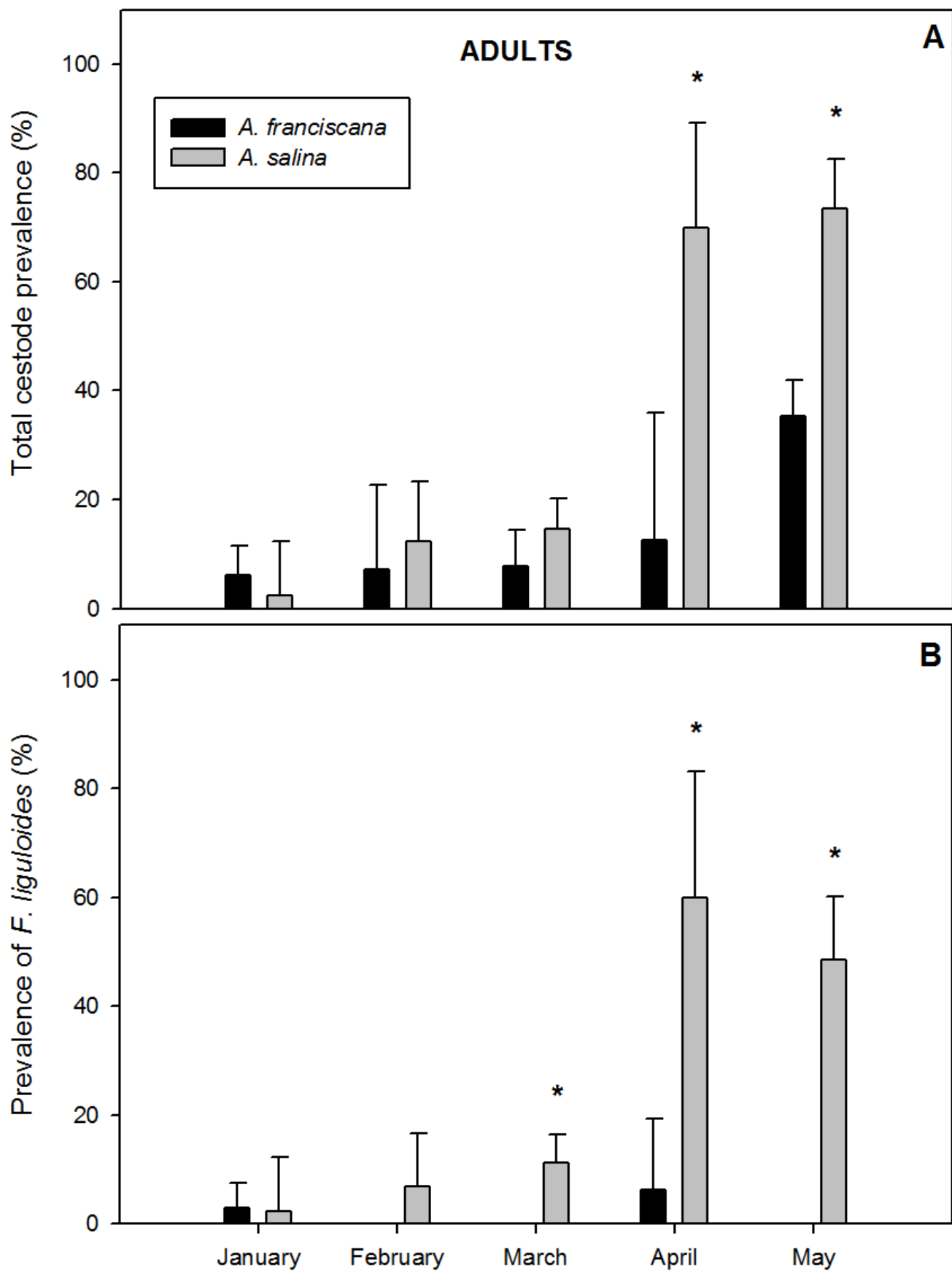
Comparative infection in juveniles of syntopic brine shrimp populations: *A. franciscana* and *A. salina* from pond CX, during the co-existence period.

(A) Total cestode prevalence, (B) Prevalence of *Flamingolepis liguloides*. Bars show upper 95% confidence intervals. * significant differences at $p < 0.05$ according to Fisher Exact tests. No cestodes were recorded in January.

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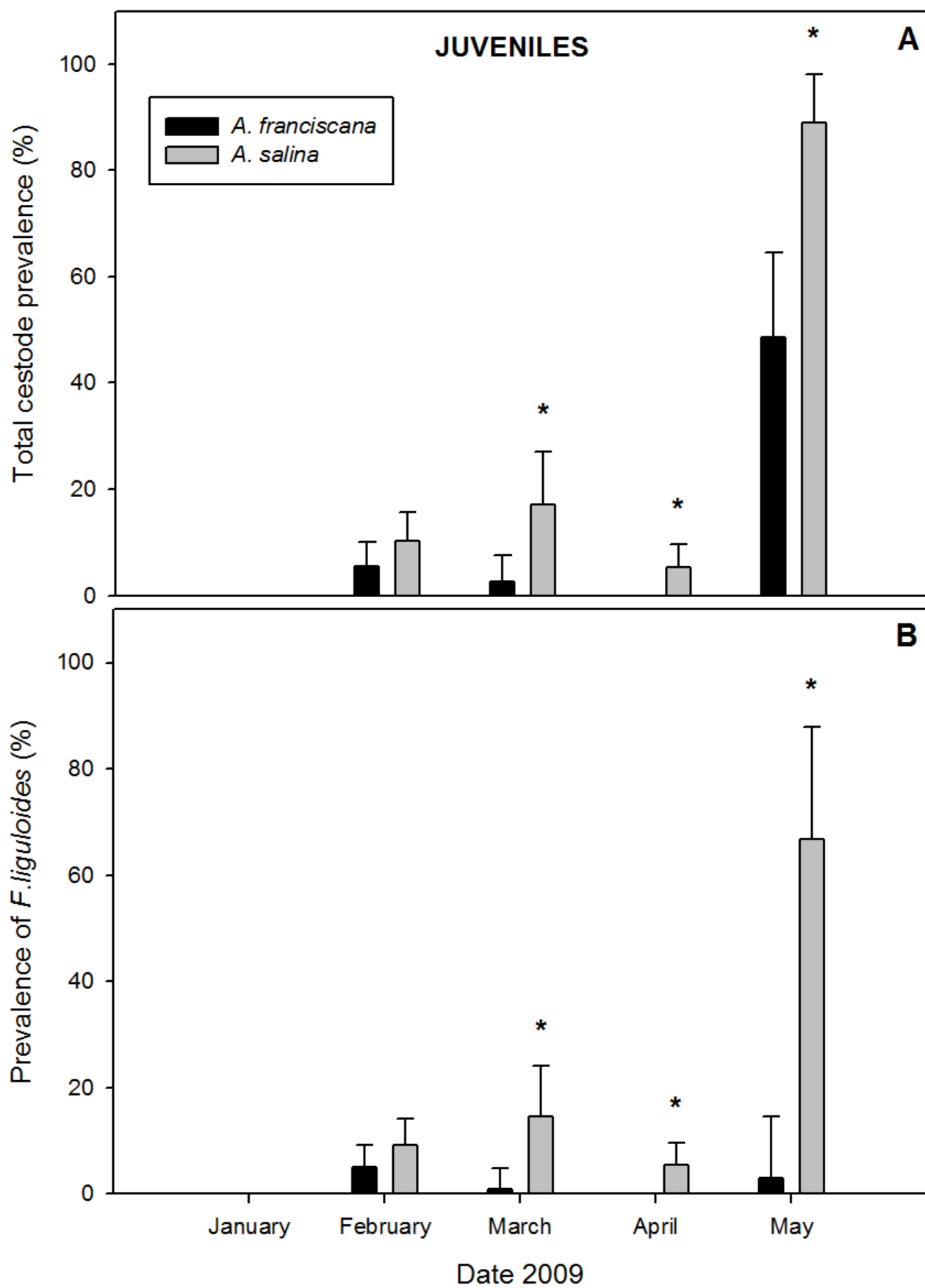
Proportion of infected adults and juveniles with red colouration for *A. salina* (AS) and *A. franciscana* (AF).
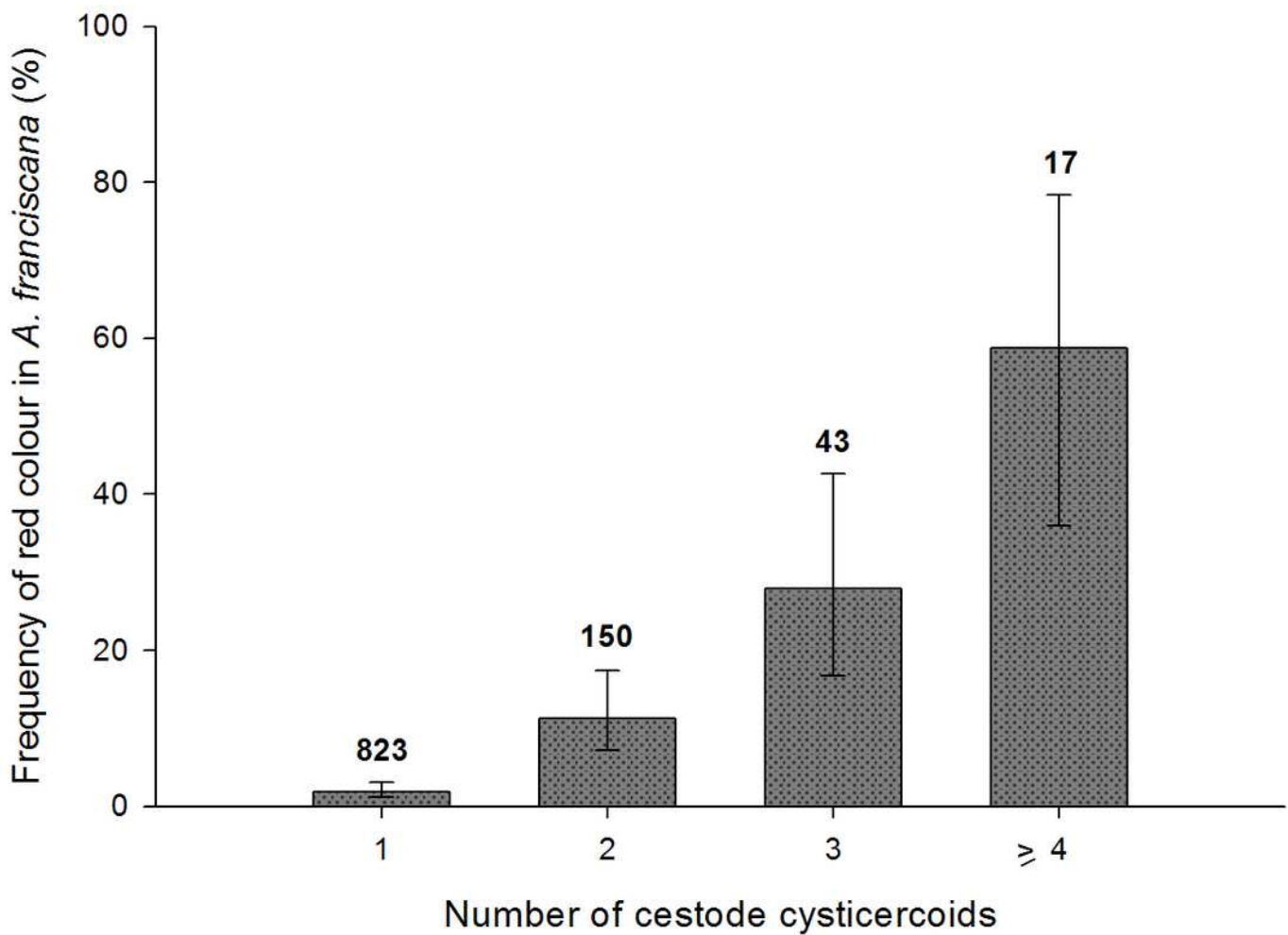
Bars show 95% confidence intervals. The total number of infected specimens in each group is shown within the columns. Red colouration was not recorded in uninfected *A. salina*, and was very rare in *A. franciscana* (absent in juveniles, 0.02% in adults). For all four categories the proportion of red shrimps is significantly higher in infected individuals (Fisher exact, $p < 0.001$).

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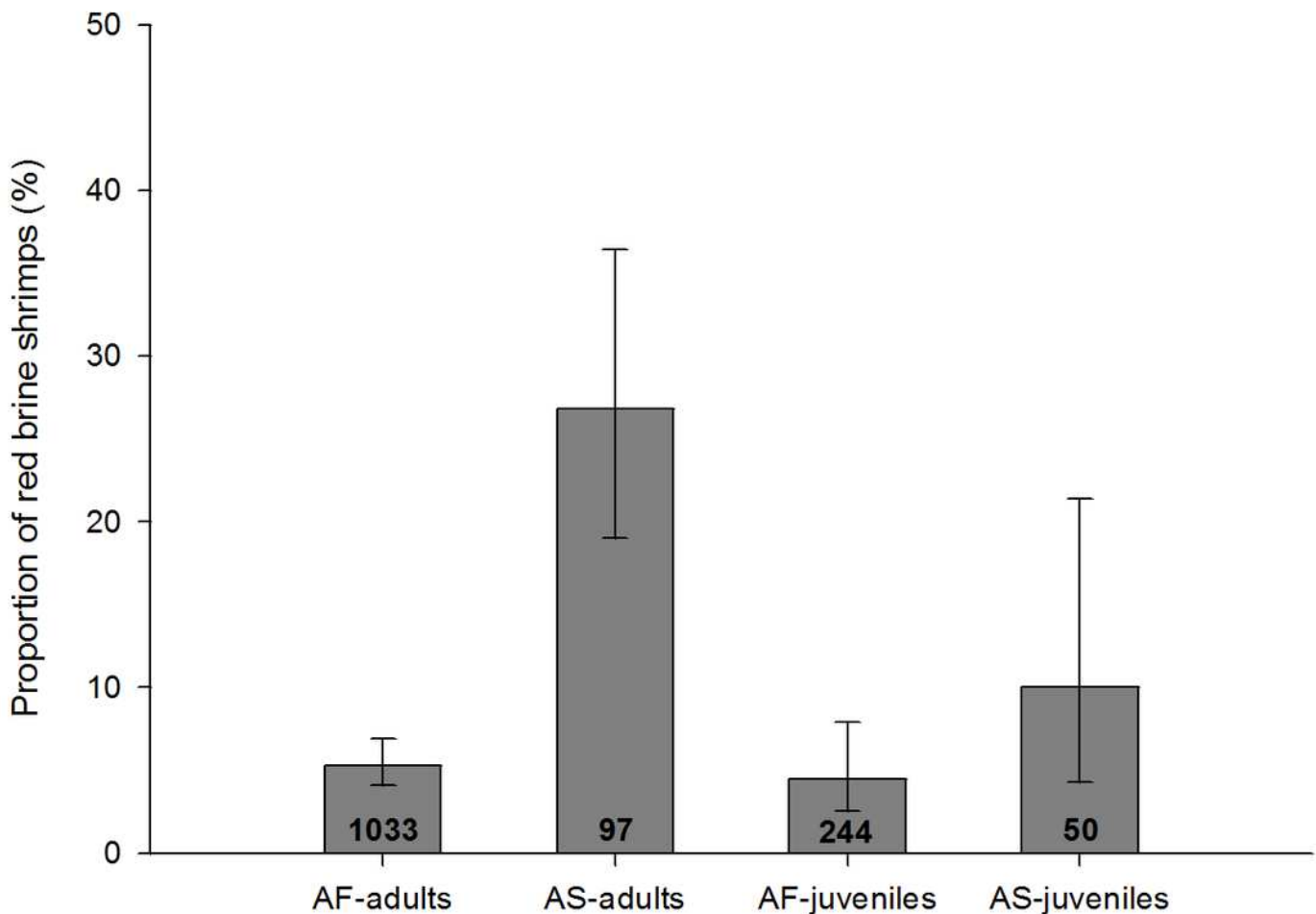
Colour pattern in relation to intensity of infection in adult *A. franciscana*.

Bars show 95% confidence intervals. The total number of infected specimens in each group is indicated.

Castration effects in adult female *A. franciscana* and *A. salina*.

Bars show 95% confidence intervals.