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

Stella Redón, Francisco Amat, Marta I. Sánchez, Andy J. Green

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 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.

2 **Authors:**

4 Stella Redón[1], Francisco Amat[1], Marta I. Sánchez[2] and Andy J. Green[2]

7 [1]Instituto de Acuicultura de Torre de la Sal (IATS-CSIC), Ribera de Cabanes s/n, 12595

8 Castellón, Spain.

9 [2]Department of Wetland Ecology, Estación Biológica de Doñana (EBD-CSIC), Américo

10 Vespucio s/n, 41092 Sevilla, Spain

14 **Corresponding author:** Stella Redón, Instituto de Acuicultura de Torre de la Sal (IATS-CSIC),

15 Ribera de Cabanes s/n, 12595 Castellón, Spain. Tel.: +34 964 31 95 00. E-mail:

16 stellarc.bio@gmail.com

27 **Introduction**

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

29 aquaculture industry and a model organism for laboratory research (e.g. in toxicology, genetics

30 or physiology). *A. franciscana* cysts, particularly originating from Great Salt Lake (USA), have

31 been exported worldwide for aquaculture, the improvement of salt extraction in salt ponds and

32 the pet trade market, facilitating the arrival and spread of *A. franciscana* outside its natural range

33 (Amat et al., 2005; Ruebhart, Cock & Shaw, 2008; Vikas et al., 2012). In the Western

34 Mediterranean, the introduction of this exotic species is provoking the extinction of native *A.*

35 *salina* (Linnaeus, 1758) and *A. parthenogenetica* Bowen & Sterling, 1978 populations (Amat et

36 al., 2005; Amat et al., 2007; Muñoz et al., 2014). The ability of *A. franciscana* to outcompete

37 other *Artemia* species rapidly in the field may be largely explained by a higher reproductive rate

38 that often allows it to eliminate native congeners within a few generations in the laboratory

39 (Browne, 1980; Browne & Halanych, 1989; Amat et al., 2007). However, parasites can also

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

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

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

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

44 tapeworms whose final hosts are flamingos, waders, grebes, ducks or gulls (Georgiev et al.,

45 2005; Georgiev et al., 2007; Vasileva et al., 2009). To elucidate the role of parasites in an

46 invasion it is important to study parasite infections in populations of native and alien host species

47 co-ocurring in the same habitat (syntopic populations), but this is difficult because native

48   *Artemia* have already disappeared from most sites where *A. franciscana* is detected.

49   Comparisons of allopatric *Artemia* populations from the southern Iberian Peninsula suggest that

50   *A. franciscana* populations have lower levels of infections by cestodes than the native sexual *A.*

51   *salina* and the clonal *A. parthenogenetica* (Georgiev et al., 2007; Georgiev et al., 2014; Sánchez

52   et al., 2013). In the present study, we take advantage of a unique opportunity to compare the

53   parasitism of *A. franciscana* and *A. salina* in the only site where these two sexual species are

54   known to coexist: the Ebro Delta salterns in north-east Spain.

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

56   and *A. parthenogenetica* in southern France, and found lower cestode diversity and abundance in

57   the invasive host. This could potentially be because the sexual invasive species can resist

58   parasites better than the clonal native one, owing to the importance of genetic recombination in

59   resisting parasites (Red Queen hypothesis: Van Valen, 1973; Moritz et al., 1991; Mee & Rowe,

60   2006). However, in the case of two sexual species, the relative effects of parasites may be more

61   similar. Given the lack of preexisting data, comparing parasite impacts in two bisexual hosts (*A.*

62   *franciscana* and *A. salina*) is of considerable interest in the context of the biological invasion.

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

64   induce changes in the physiology, behaviour or appearance of intermediate hosts ("host

65   manipulation"), rendering them more vulnerable to predation and facilitating transmission to

66   final hosts (Barber, Hoare & Krause, 2000; Moore, 2002; Poulin, 2006). In native Mediterranean

67   *Artemia* populations, cestode infections are associated with a reddish colour, positive phototaxis,

68   gigantism, longer life span, reduced fecundity and higher levels of lipids and glycogen (Thiéry,

69   Robert & Gabrion, 1990; Amat et al., 1991; Robert & Gabrion, 1991; Sánchez et al., 2006;

70   Sánchez, Georgiev & Green, 2007; Amarouayache, Derbal & Kara, 2009; Sánchez et al., 2009a).

71   The pathogenic impact of endemic cestodes on the alien *A. franciscana* remains unclear,

72   particularly when it co-occurs with native congeners. If *A. franciscana* is less affected by

73   cestodes, this may help explain its invasion success by aiding it to outcompete native hosts.

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

75   *salina* in a salt pond where they coexisted. We tested the hypothesis that *A. franciscana* is less

76   susceptible to native cestodes than *A. salina*, leading to a competitive advantage. Secondly, we

77   compared the infections between this *A. franciscana* population that is coexisting with a native

78   host (*A. franciscana*-syntopic population), and an *A. franciscana* population from a neighbouring

79   pond where there are no congeners, in order to explore if there is a negative relationship between

80   community diversity and disease risk (Schmidt & Ostfeld, 2001; Keesing, Holt & Ostfeld, 2006;

81   Johnson &Thieltges, 2010). Thirdly, we analyzed the consequences of infection by different

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

83   predicted fewer 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) along

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

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

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

91   salterns supported a tetraploid parthenogenetic population of *Artemia* (Amat et al., 1995), but

92   this native taxon has not been 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

95   2009 to January 2010, when the native *A. salina* was in coexistence with *A. franciscana*. 1) Pond

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

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

98   Samples were collected monthly from January 2009 to January 2010 (salinity S range = 45 - 260

99   g/L, mean ± s.e. = 114.6 ± 18.35; temperature T range = 5 - 32ºC, mean ± s.e. = 18.1 ± 2.37). 2)

100   Pond 4 was sampled monthly from January to March 2009, then again in January 2010 (S range

101   = 125 - 150 g/L, mean ± s.e. = 132.5 ± 5.95; T range = 8.5 – 12ºC, mean ± s.e. = 10.9 ± 0.83).

102   In order to increase the sample size for infected shrimps, additional samples of *A. franciscana*

103   collected on other dates and in other ponds (details in S. Redón, AJ Green, BB Georgiev, GP

104   Vasileva, F Amat, 2015 submitted) were used when considering the influence of cestodes on

105   colouration and reproductive activity of this host species.

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

107   nets and transported alive to the laboratory. Living *Artemia* individuals, anaesthetized with a few

108   drops of distilled water saturated with chloroform, were examined under a stereomicroscope and

109   juveniles and adults were separated. Juveniles are immature specimens with sexual segments

110   (ovisac or hemipenis) that are not completely developed. Juveniles and adults were identified to

111   species after Amat (1985), Hontoria & Amat (1992a) and Hontoria & Amat (1992b). The

112   proportion of juveniles varied over time (Fig. S1) confirming that both species had multiple

113   generations per year (Amat et al., 2007). Both juveniles and adults were sexed (Amat, 1985).

114   Adult females with empty ovisac and no signs of functional ovaries were classified as castrated

115   and those with embryos, naupliae, or cysts filling the ovisac, or ovulating (oocites moving along

116   the ovaries or filling the oviducts) were classified as ovigerous (Fig. S3). The colour of adults

117   and juveniles was assigned to three categories: dark-red, light-red and not-red (Fig. S2). This

118   research was conducted under a permit from the Ebro Delta Natural Park office provided to FA.

119   *Parasite identification*

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

121   cestode cysticercoids, until a total of approximately 400 individual shrimps (when available) was

122   reached, including juveniles and adults. After observations of the cysticercoids *in situ*, each

123   infected specimen was prepared in a temporary glycerol mount and examined under a compound

124   microscope. Identification was based on Georgiev et al. (2005) and Vasileva et al. (2009). More

125   details of sampling protocols are provided by S Redón, AJ Green, BB Georgiev, GP Vasileva, F

126   Amat, submitted.

127   *Quantitative analysis of cestode infection and statistics*

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

129   proportion of infected individuals in the host population), abundance (MA: mean number of

130   cysticercoids for the total number of shrimps examined) and intensity (MI: mean number of

131   cysticercoids in infected shrimps) were calculated for the overall infection and for each parasite

132   species (terminology following Bush et al., 1997). Also, we quantified species richness (SR:

133   mean number of cestode species present in each *Artemia* individual) and the species richness in

134   infected specimens (SRinfected).

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

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

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

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

139   parasites, the infection intensity and SR between host species, separately for adults and

140   juveniles. Differences in the prevalence of cestodes between host species were analyzed with

141   Fisher Exact tests.

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

143   We compared the parasite infections in *A. franciscana* in a syntopic population (presence of *A.*

144   *salina*, pond CX) with those in a single species population sampled simultaneously (pond 4).

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

146   *franciscana* between ponds on the same sampling day during three months (January, February

147   and March 2009), separately for adults and juveniles.

148   *Parasite castration effects*

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

150   ponds; S. Redón, AJ Green, BB Georgiev, GP Vasileva, F Amat, submitted), we compared the

151   proportion of infected and uninfected females that were castrated. The castration effect of

152   cestodes in *A. franciscana* was evaluated by Wilcoxon tests for paired samples, comparing the

153   proportions of castrated shrimps within a given sample, thus avoiding any non-independence of

154   observations of different individuals within the same sample. In contrast, Fisher Exact tests were

155   applied for *A. salina*, because the small number of samples with this species obliged us to pool

156   them and treat each individual shrimp as an independent observation. Since castration was never

157   recorded in uninfected female *A. salina*, this pooling was unlikely to bias the observed levels of

158   castration.

159   *Effects of parasites on colour*

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

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

162   between infected and non-infected specimens with Fisher Exact tests. The same test was

163   employed to analyze colour-effects between host sexes and host species for both adults and

164   juveniles. In addition, to test the influence of infection intensity on colour, Mann-Whitney U

165   tests were applied to compare the cestode intensity between infected individuals that were red

166   and those that were not. All statistical analyses were carried out using SPSS 15.0 for Windows

167   (SPSS Inc., Chicago, IL, USA).

168   **Results**

169   *Comparing parasite loads in A. franciscana and A. salina in syntopic populations*

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

171   from January to May 2009, and September 2009 to January 2010 (Fig. 2), whereas *A.*

172   *franciscana* was present all year long. Nine cestode species were detected in *A. franciscana*, and

173   seven of these in *A. salina* (Table 1). These included four species whose adults are parasitic in

174   flamingos (*Flamingolepis liguloides*, *F. flamingo*, *Gynandrotaenia stammeri* and

175   *Gynandrotaenia* sp.), three in shorebirds (*Anomotaenia tringae*, *A. microphallos* and *Eurycestus*

176   *avoceti*), one in gulls (*Wardium stellorae*) and one in shelducks (*Fimbriarioides tadornae*).

177   Adult brine shrimps

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

179   *salina* and 19.1% of *A. franciscana* were parasitized by cysticercoids (Table 1). Cestodes were

180   detected in all five months of co-existence (Fig. 3). For overall infection, *A. salina* had a higher

181   prevalence than *A. franciscana* (Table 1), with statistically significant differences in April and

182   May (Fig. 3A).

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

184   in *A. franciscana*, and was significantly higher in *A. salina* in April and May (Mann-Whitney U

185 tests, $p < 0.05$). Total cestode intensity ranged from 1 to 1.84 in *A. salina* and 1 to 1.19 in *A.*

186 *franciscana*, and was significantly higher in the former in May ($p < 0.001$). The relative

187 abundance of cestode species varied between hosts. *F. liguloides* was the most prevalent and

188 abundant parasite in *A. salina* (Table 1), and its prevalence and abundance were significantly

189 lower in *A. franciscana* in March, April and May (Fig. 3B). In May, the prevalence and

190 abundance of *A. tringae* were also significantly higher in *A. salina* (41.2% and 0.471 ± 0.08,

191 respectively) than *A. franciscana* (11.6% and 0.126 ± 0.03). Except in January, *E. avoceti* was

192 the most prevalent and abundant parasite in *A. franciscana* (Table 1). No significant differences

193 in the intensity of individual cestode species were recorded (Table 1).

194   *A. salina* had a higher SR of cestodes (Table 1), being significantly higher in April (0.70

195 ± 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 ±

196 0.04, $p < 0.001$). In May, the SR of infected specimens was also significantly higher for *A.*

197 *salina* (1.52 ± 0.09 *vs.* 1.12 ± 0.04, $p < 0.001$).

198 Juvenile brine shrimps

199 A total of 1,282 juveniles were examined in samples with both species present. Of these, 9.5% of

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

201 detected in all months of co-existence except January (Fig. 4). For the overall infection,

202 prevalence and abundance were significantly higher in *A. salina* from March to May (Fig. 4A).

203 No significant differences were detected in intensity between host species (Table 1).

204   Prevalence and abundance of *F. liguloides* were significantly higher in *A. salina* from

205 March to May (Fig. 4B). Prevalence and abundance of *A. tringae* were also significantly higher

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

207 higher for *A. salina* in March (0.18 *vs.* 0.04; $p = 0.001$), April (0.05 *vs.* 0.00; $p < 0.001$) and May

208 (1.56 *vs.* 0.63; $p = 0.003$). However, no significant differences were detected for SR of infected

209 specimens.

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

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

212 each of three months in pond CX where it was coexisting with *A. salina* (AF-syntopic) than in

213 pond 4 where it was the only *Artemia* species present (AF-single), with statistically significant

214 differences in March (Table 2). Prevalence was significantly higher in AF-syntopic for *F.*

215 *liguloides* and *E. avoceti* in January and March, respectively (Table 2). SR was significantly

216 higher in AF-syntopic in March (Table 2). No significant differences were detected for infection

217 intensity.

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

219 significantly higher in AF-syntopic in March (as for adults), but significantly lower in AF-

220 syntopic in January (when no infected juveniles of either *Artemia* species were recorded in the

221 syntopic population). The abundance of *F. flamingo* in March was also significantly higher for

222 AF-syntopic (Table 3).

223 *Influence of parasites on host colouration*

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

225 in both adults and juveniles (Fig. 5, Table 4). Among infected adults, red colouration was

226 significantly more frequent in *A. salina* than *A. franciscana* (Table 4). There were no differences

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

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

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

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

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

232   = 11280, *p* < 0.001) and *A. salina* (2.08 ± 0.20 when red, 1.31 ± 0.07 when not; *U* = 487, *p* <

233   0.001). A similar result was obtained for infected juvenile *A. salina* (3.8 ± 0.58 when red, 1.16 ±

234   0.06 when not; *U* = 4, *p* < 0.001). Owing to the larger sample size, the positive correlation

235   between infection intensity and probability of red colouration was particularly clear in *A.*

236   *franciscana* adults (Fig. 6), for which the same pattern was apparent for the dominant cestode

237   species, *E. avoceti* (results not shown).

238   Where only a single species of cestode was present (single infections), there was a

239   significant redness effect in the case of six species for *A. franciscana* adults, three species for *A.*

240   *franciscana* juveniles and two species for *A. salina* adults (Table 4). In most of these cases, the

241   presence of a single cysticercoid was enough to cause a significant effect (Table 4). For *A.*

242   *salina*, no colour-effects were observed for single infections of the most abundant cestode, *F.*

243   *liguloides* (Table 4).

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

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

246   with 9.5% red) (Fisher Exact test, *p* = 0.001). No significant differences between host species in

247   colour-effects were detected for other cestode species, although sample sizes for *A. salina* were

248   small (Table 4).

249   *Cestodes and host castration*

250   In *A. franciscana* adults, a higher proportion of infected females were castrated (i.e. with empty

251   ovisac and no signs of functional ovaries, Fig. S3) than those which were not infected females

252   (Fig. 7, Table 5).

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

254   parasite species (i.e. single infections, Table 5), and of these most were infected with *E. avoceti*

255   (64.3%) or *F. liguloides* (19%). Castration rates in females infected only by *E. avoceti* or only by

256   *F. liguloides* were significantly higher than those in uninfected females (Table 5). For *E. avoceti*,

257   the probability of castration clearly increased with the intensity of infection, and 35.7% of

258   females with ≥ 3 cysticercoids were castrated ($p = 0.001$). Unlike *E. avoceti*, infection with a

259   single *F. liguloides* cysticercoid had a significant castration effect ($p = 0.004$). Of female *A.*

260   *franciscana* infected by more than one cestode species (co-infections) (N = 57), 35.1% were

261   castrated. Among these castrated females (N = 20), *E. avoceti* was present in 18 individuals and

262   *F. flamingo* in seven.

263   For *A. salina*, castration was only recorded in infected females (Fig. 7, Table 5).

264   Castration effects were significant for single infections by *F. liguloides* or by *A. tringae* (Table

265   5). If we include females with small broods (<10 eggs in the ovisac) and no oocytes migrating in

266   the ovaries, the proportion of female *A. salina* with limited fecundity when infected with *F.*

267   *liguloides* increases from 35.7% to 57.1%.

268

269   **Discussion**

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

271   closely related branchiopod crustaceans. The native *A. salina* generally had more cestodes than

272   the alien *A. franciscana*. When coexisting with the native, the alien had higher rates of infection

273   than when alone. Although the cestodes were shown to have important effects on the fecundity

274   and colour of both host species, these effects were stronger in the native species.

275

276 Comparing infections in syntopic *A. franciscana* and *A. salina*: how important is co-evolution?

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

278 understand the role of parasites in biological invasions (Dunn, 2009; Kelly et al., 2009; Dunn et

279 al., 2012). The present work sheds light on the role of endemic cestodes in competitive

280 interactions between native and alien sexual *Artemia*. Cestode parasitism differed clearly

281 between invasive and native brine shrimps under the same environmental conditions and at the

282 same time. Cestode species richness and the prevalence of several species were lower in *A.*

283 *franciscana*, and the difference was especially striking for *F. liguloides* (Figs. 3, 4). These results

284 are consistent with studies of populations in the southern Iberian Peninsula which have found *A.*

285 *franciscana* to have low infection rates throughout the annual cycle. In summer, Georgiev et al.

286 (2007) found lower levels of cestode infections in three *A. franciscana* populations than in an

287 allopatric *A. salina* population. Studies throughout the annual cycle of *A. franciscana* in Cadiz

288 Bay and of *A. salina* in Almería also found that the invasive species had fewer infections

289 (Sánchez et al., 2013; Georgiev et al., 2014). Our results for syntopic populations indicate that

290 these earlier results are not explained by other differences between these allopatric populations

291 (e.g. in environmental conditions, or in the density of birds). A previous study in syntopic

292 populations in Aigües-Mortes in France showed that *A. franciscana* had fewer cestodes than

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

294 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

298 several native cestode species such as *F. liguloides*. Different capacities to infect the new host

299   may be the result of varying host-parasite co-evolutionary history. The lower infection of *F.*

300   *liguloides* in *A. franciscana* may be explained by a strong immune response of the host against

301   this parasite, given the restricted distribution of flamingos in North America which barely

302   overlaps with the natural range of *A. franciscana* (Muñoz et al., 2013). There are no flamingos in

303   the western USA, from where *A. franciscana* has been exported around the world. Observations

304   of dead cysticercoids of *F. liguloides* in *A. franciscana* support a strong immune response in this

305   host (Georgiev et al., 2014). In contrast, the high prevalence of *E. avoceti* recorded in *A.*

306   *franciscana* in our study may reflect a shared coevolutionary history, since *E. avoceti* occurs in

307   shorebirds in North America (Clark, 1954). *A. franciscana* does not appear to be especially

308   resistant to cestodes in its native range, since the total prevalence of cestodes in Great Salt Lake,

309   USA was much higher than in the Ebro Delta and other parts of the invasive range (Redón et al.,

310   2015). Our results are consistent with previous studies that have found parasites of native hosts

311   to have no or a limited capacity to spread to non-indigenous congeners (Dunn & Dick, 1998;

312   Torchin, Byers & Todd, 2005; Genner, Michel & Tood, 2008).

313

314   How does coexistence of both species influence infection parameters in *Artemia franciscana*?

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

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

318   imported cysts (dormant eggs) free of native cestode parasites, so "spillover" is not expected in

319   this system. The low levels of parasitism recorded in *A. franciscana* indicate that it is a poor or

320   incompetent reservoir for native cestodes, making "spillback" to native *Artemia* unlikely. We

321   might expect a parasite dilution effect of the invader to the benefit of the native host which

322 should be subjected to lower disease transmission when mixed with incompetent, alien hosts

323 (Keesing, Holt & Ostfeld, 2006; Hall et al., 2009; Johnson & Thieltges, 2010). A reduced

324 infection rate by native trematodes was demonstrated experimentally in native freshwater snails

325 *Potamopyrgus antipodarum* exposed together with the invader *Lymnaea stagnalis* (Kopp &

326 Jokela, 2007). Given the strong effects of cestodes on *A. salina*, such a "dilution effect" could

327 increase the possibility of local persistence of *A. salina* in the presence of the invader (Hatcher,

328 Dick & Dunn, 2006).

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

330 assess the strength of any dilution effect on this species. However, the levels of cestode

331 prevalence we recorded in the presence of *A. franciscana* were not unusually low compared to

332 studies of *A. salina* elsewhere in the absence of the invader (Table S1). Likewise, Sánchez et al.

333 (2012) found no evidence of a dilution effect on *A. parthenogenetica* when coexisting with *A.*

334 *franciscana*, since the total prevalence in *A. parthenogenetica* was high (c.70%).

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

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

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

338 temporal dynamics of infection in *A. franciscana* in pond CX (with both *Artemia* species) seem

339 to depend on the proportion of native hosts in the *Artemia* population. Thus, total prevalence

340 increased from January to May 2009 (coinciding with the abundance of *A. salina*), then declined

341 from June to December when *A. franciscana* was the dominant species (Fig. S4). In contrast, in

342 pond 4 (without *A. salina*), higher prevalences were recorded in July and August during 2007

343 and 2008 (S Redón, AJ Green, BB Georgiev, GP Vasileva, F Amat, submitted). Since *A.*

344 *franciscana* is a poor reservoir (i.e. the cestodes may not circulate effectively in them), adding *A.*

345   *salina* to the community might increase the prevalence of the infection in the alien host because

346   the added host is a better reservoir (Keesing, Holt & Ostfeld, 2006, Hatcher, Dick & Dunn,

347   2006). Since *Artemia* are only intermediate hosts, such an explanation would require final avian

348   hosts to be relatively faithful to individual ponds, so that birds that become infected in the pond

349   with *A. salina* are more likely to release cestode eggs there than in other ponds. Another possible

350   and not mutually exclusive explanation for our results is that the final avian hosts are more

351   attracted to ponds holding *A. salina* because this is their preferred prey, leading to greater

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

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

354   shown in our study, they are less likely to have the red colour preferred by birds (Sánchez et al.,

355   2009b). In turn, this suggests that the generally low infection rates in *A. franciscana* may be

356   accounted for not only by a greater resistance to parasites than native species, but also by a

357   tendency for final hosts (birds) to avoid invaded salt ponds, reducing levels of parasite

358   circulation. A study of how waterbird use differs between salt ponds with native or alien shrimp

359   is required.

360

361   Castration and colour effects in native and alien hosts

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

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

364   2002; Thomas, Adamo & Moore, 2005; Médoc, Bollache & Beisel, 2006). Parasitic castrators

365   benefit by diverting host resources away from reproduction, allowing faster growth and earlier

366   arrival at the infective stage, or an increase in host lifespan, thus increasing opportunities for

367   transmission (Hurd, Warr & Polwart, 2001). *A. parthenogenetica* infected with *F. liguloides* live

368   longer than uninfected ones (Amat et al., 1991), and this increased host longevity may ensure the

369   complete development of the infective stage from oncosphere to ripe cysticercoid (see Redón et

370   al., 2011), ready to be transmitted to final hosts.

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

372   stronger effects in native *A. salina*. Despite the smaller sample size, for *A. salina* we could

373   confirm a castrating effect for *A. tringae* and *F. liguloides.* The latter species was already known

374   to have a strong castrating effect on *A. salina* (Amarouayache, Derbal & Kara, 2009) and *A.*

375   *parthenogenetica* (Amat et al., 1991; Sánchez et al., 2012). In *A. franciscana*, we found

376   significant castration effects for *F. liguloides* and *E. avoceti*, with a stronger effect by the former

377   (a single cysticercoid of *F. liguloides* is able to castrate, whereas two or more are needed for *E.*

378   *avoceti*). This is the first time that a castration effect has been demonstrated for dilepidid

379   cestodes in *Artemia*, and suggests these effects are widespread and not limited to *F. liguloides*.

380   Our results have important implications for the use of *A. franciscana* in aquaculture, since they

381   show that cestode infections reduce host fecundity, even in the introduced range. This suggests

382   that cyst production in sites within the introduced range where cysts are harvested for

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

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

385   range, and the first to consider the effects in *A. salina* in detail. The association of red

386   colouration with cestode infection is well known for native *A. parthenogenetica*, especially for

387   *F. liguloides*, and appears to be due to parasite manipulation associated with the preference that

388   final hosts have for predating red *Artemia* (Sánchez et al., 2009b; Sánchez et al., 2012).

389   However, it is also possible that this altered pigmentation is caused by a host immune response

390   against infection (e.g. given the antioxidant function and immunostimulating properties of

391   carotenoids) rather than a specific parasitic strategy to ensure transmission. The red colour of

392   cestode-infected *A. parthenogenetica* is partly due to carotenoids (Amat et al., 1991; Sánchez et

393   al., 2006) and carotenoids seem to play an important role in immune defence by providing free

394   radical scavengers against cestode infection (van der Veen, 2005). Cornet, Biard & Moret (2007)

395   found a positive relationship between carotenoid concentration in haemolyymph and immune

396   parameters (those evolved in the prophenoloxidase cascade) in the crustacean *Gammarus pulex*,

397   suggesting that carotenoids can help to reduce the costs of immunity by limiting self-harming. A

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

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

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

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

402   *franciscana*, whether considering co-infections or only single infections. This further suggests

403   that the invasive species has greater resistance to cestode infections. Red colouration in *A. salina*

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

405   of *A. tringae* was stronger than in *A. franciscana*. Surprisingly, we could not detect any effect of

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

407   parasite than the asexual *A. parthenogenetica*, as predicted by the Red Queen hypothesis.

408   However, red colouration may be of less value to increase transmission to the filter-feeding

409   flamingos that are final hosts of *F. liguloides*, than to shorebirds which are visual predators and

410   final hosts of *E. avoceti* and *Anomotaenia* spp.

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

412   dilepidids (*E. avoceti*, *A. tringae*, and *A. microphallos*), and to a lesser extent with flamingo

413   parasites (*F. liguloides* and *F. flamingo* and *G. stammeri*), a finding in line with the higher

414   prevalence and castration effects we recorded for dilepidids. There was a strong positive

415   correlation between red-colour and overall infection-intensity, as previously observed for *A.*

416   *parthenogenetica* (Sánchez et al. 2006). Ours is the first study to compare redness effects

417   separately in juvenile and adult *Artemia*. For *A. franciscana* juveniles, we found a redness effect

418   for three cestode species, indicating that cestodes manipulate host colour even in early stages of

419   host development. There is a need for detailed research into the nature and function of the

420   pigments causing red colouration in different *Artemia* species and life stages parasitized by

421   different cestodes.

422   Previous studies in other host-parasite systems have also found native parasites to have

423   less pathological effects in alien hosts than in native hosts (Bauer et al., 2000; Cornet, Sorci &

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

425   (2010) found that a local parasite *Pomphorhynchus laevis* had less ability to infect and induce

426   pathogenic effects in invasive than in native gammarids.

427

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

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

430   introduction (see also S. Redón, AJ Green, BB Georgiev, GP Vasileva, F Amat, submitted).

431   However, it is not clear that all the cestodes parasitizing native *Artemia* in the Mediterranean

432   region will be able to persist if all the native populations are replaced by the American species.

433   Coextinctions (i.e. the loss of one especies as a result of the extinction of the species it depends

434   on; Dunn et al., 2009) seem likely in our host-parasite system, especially at a local scale of

435   individual ponds. Cysticercoids have to be able to establish, survive and grow until reaching an

436   infective-stage in brine shrimps, and then reach avian final hosts to complete the parasite´s cycle.

437   It is possible that some cestodes will not be able to infect this new host at a high enough rate or

438   to reach their final hosts with a sufficiently high frequency (according to the very low

439   prevalences and the weaker manipulation observed) to ensure the survival of a viable

440   metapopulation. When introduced species become hosts for native parasites it does not

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

442   The reduced levels of infection and reduced castration effects in *A. franciscana* suggest that

443   cestodes may not be able to infect or manipulate the alien host to a sufficient extent to ensure

444   viability. The reduced redness suggests that cestodes are less likely to reach final hosts (through

445   bird predation) when infecting alien *Artemia*, and in turn that the value of salterns for waterbird

446   conservation may be reduced by the invasion. Parasites are an important component of food

447   webs (Lafferty, Dobson & Kuris, 2006, Lafferty et al., 2008) and ecosystem functioning

448   (Hudson, Dobson & Lafferty, 2006), so loss of cestodes may have an impact on the stability of

449   hypersaline ecosystems.

450

451   ***Conclusions***

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

453   ways, suggesting that native cestodes are poorly adapted to these novel hosts. They cause

454   infections of higher prevalence and species richness in the native host. For a given infection

455   intensity, they cause a greater impact on host fecundity, and a greater change in colouration,

456   likely to translate into a higher predation rate by birds. Therefore, cestodes can influence

457   competition between *Artemia* species and are likely to help *A. franciscana* to outcompete native

458   species in the Mediterranean region and other parts of the world. In turn, the viability of these

459   cestode populations across broad scales, where waterbird populations interact with different

460   hypersaline ecosystems, is threatened by the loss of native *Artemia* which may act as a "source"

461   for cestodes whereas *A. franciscana* populations may act as a "sink".

462

463   **Acknowledgements**

464   We are grateful to the Parc Natural del Delta de l´Ebre staff, as well as to the La Trinitat salterns

465   staff, who provided facilities to work in the natural park and the salt ponds. The authors would

466   like to thank M. Ghomari and M. Maccari for their help with field sampling. Boyko Georgiev

467   and an anonymous referee helped to improve an earlier version of this manuscript.

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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.

Cestode species		Host - adults		Host - juveniles	
		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

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

Parasite infections from a population without co-ocurrence of congeners (pond 4, AF-single) and one in co-existence with *A. salina* (pond CX, AF-syntopic) 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.

Cestode species		January 2009			February 2009			March 2009		
		AF-single N =963, S =125g/l	*AF-syntopic* (75.9%) N =132, S =56g/l	*p* value	*AF-single* N =244, S =130g/l	*AF-syntopic* (32.9%) N =28, S =65g/l	*p* value	*AF-single* N =186, S =150g/l	*AF-syntopic* (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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Comparative cestode infection in juvenile *A. franciscana* from single and syntopic populations.

Parasite infections from a population without co-occurrence of congeners (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.

Cestode species		January 2009				February 2009				March 2009		
		AF-single N =168	AF-syntopic (65.7%) N =134	p value		AF-single N =67	AF-syntopic (50.4%) N =179	p value		AF-single N =248	AF-syntopic (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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Effects of infection status on colouration of *Artemia*.

Data are presented for adults and juveniles of *A. franciscana* and *A. salina* from Ebro delta salterns. The total number of individuals examined (including males and females) is given in parentheses. See Table 1 for abbreviations of parasite species. Different superscript letters denote significant differences between *Artemia* species of the same age group in the proportion of red individuals (Fisher Exact test, $p < 0.05$). Asterisks indicate a significant difference in the proportion of red individuals with the uninfected group (Fisher Exact test, *$p < 0.05$, **$p < 0.001$). † indicates cases where a single cysticercoid of that species was enough to cause a significant increase in the probability of redness (Fisher Exact test, $p < 0.05$).

	Adults		Juveniles	
	A. franciscana	*A. salina*	*A. franciscana*	*A. salina*
	% Red	% Red	% Red	% Red
Uninfected individuals	0.02% (8260)	0% (325)	0% (8658)	0% (507)
Infected individuals	5.3%**[a] (1033)	26.8%**[b] (97)	4.5%** (244)	10%** (50)
Infection status				
Single infections (1 species)- Total	4.2%**[a] (931)	12.2%**[b] (74)	4.1%** (221)	0% (41)
Infected only by FL	2.3%* (86)	0% (47)	0% (32)	0% (35)
Infected only by FF	1.3%* (79)	0% (2)	5.3%** (114) †	0% (5)
Infected only by WS	0% (26)	0% (3)	0% (2)	-
Infected only by FT	0% (12)	-	0% (7)	-
Infected only by EA	4.2%** (620) †	12.5%* (8) †	2.3%* (42) †	0% (1)
Infected only by AT	13.6%**[a] (22) †	72.7%**[b] (11) †	-	-
Infected only by AM	18.2%** (33) †	-	66.7%** (3) †	-
Infected only by GS	2.3%* (43) †	-	0% (9)	-
Infected only by GSP	0% (7)	0% (3)	0% (2)	-
Multiple infections (≥ 2 species)	15.7%**[a] (102)	73.9%**[b] (23)	8.7%**[a] (23)	55.6%**[b] (9)

Castration effects associated with cestode infection in *Artemia*.

Data are presented for adult females of the alien *A. franciscana* and native *A. salina*. *N*, number of females examined. See Table 1 for abbreviations of parasite species. Asterisks indicate significant differences in the proportion of castrated females with uninfected females of the same *Artemia* species (*p < 0.05, **p < 0.001). Wilcoxon Signed Rank tests were conducted to compare the proportions of uninfected and infected *A. franciscana* that were castrated in different samples, for total infections and for those infected only by EA. Fisher Exact tests were conducted for other taxa after pooling samples (see methods for details).

	A. franciscana		*A. salina*	
	N total	% castration	N total	% castration
Uninfected females	3267	6.1%	111	0%
Infected females - Total	553	11.2%*	40	22.5%**
Single infections (1 species) - Total	496	8.5%*	29	20.7% **
Infected only by FL	32	25%*	14	35.7% **
Infected only by FF	28	7.1%	1	0%
Infected only by WS	14	0%	1	0%
Infected only by EA	362	7.5%*	5	0%
Infected only by AT	9	0%	5	20%*
Infected only by AM	22	4.5%	0	-
Infected only by GS	21	14.3%	0	-
Infected only by GSP	1	100%	3	0%

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Geographical situation of the study 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

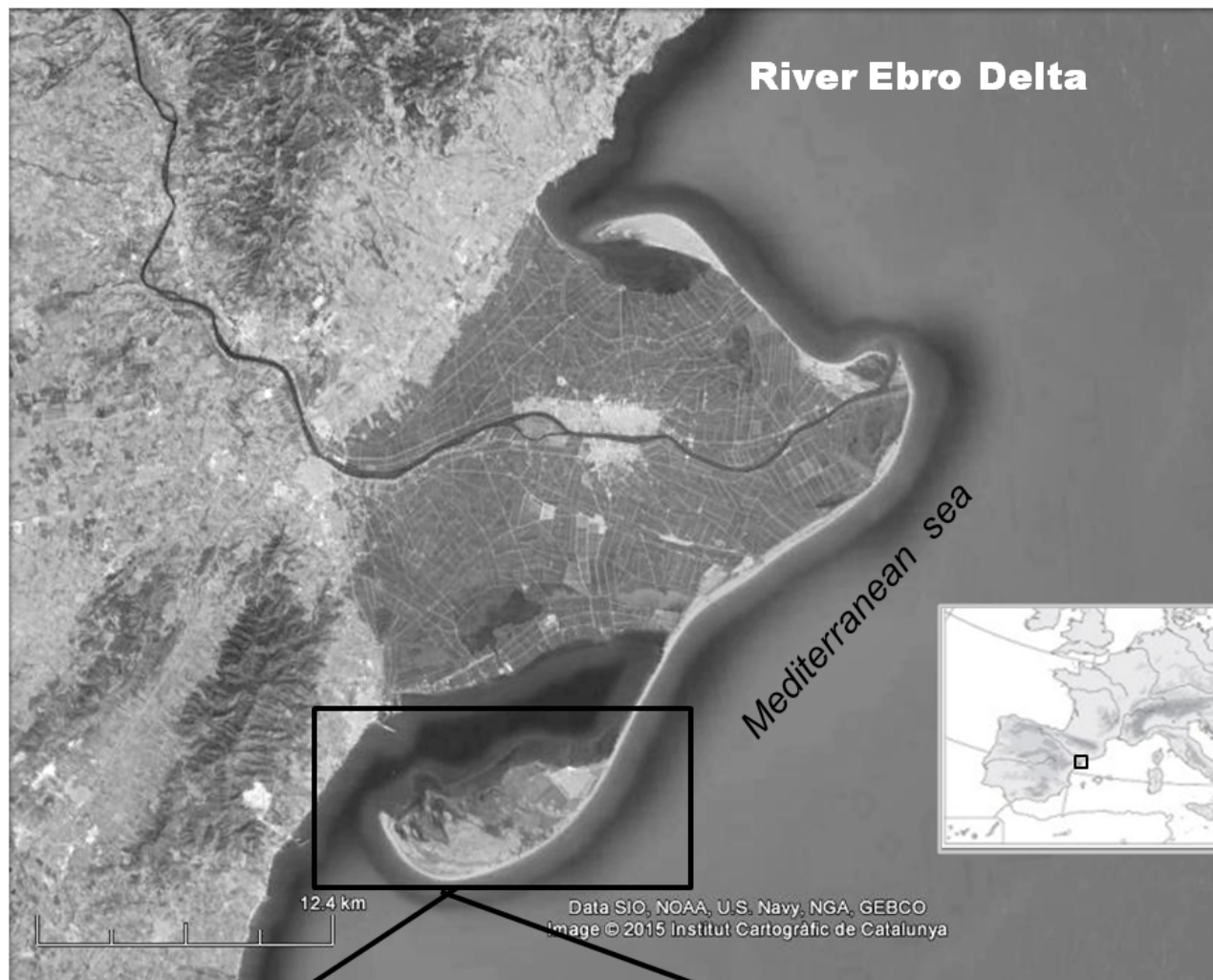
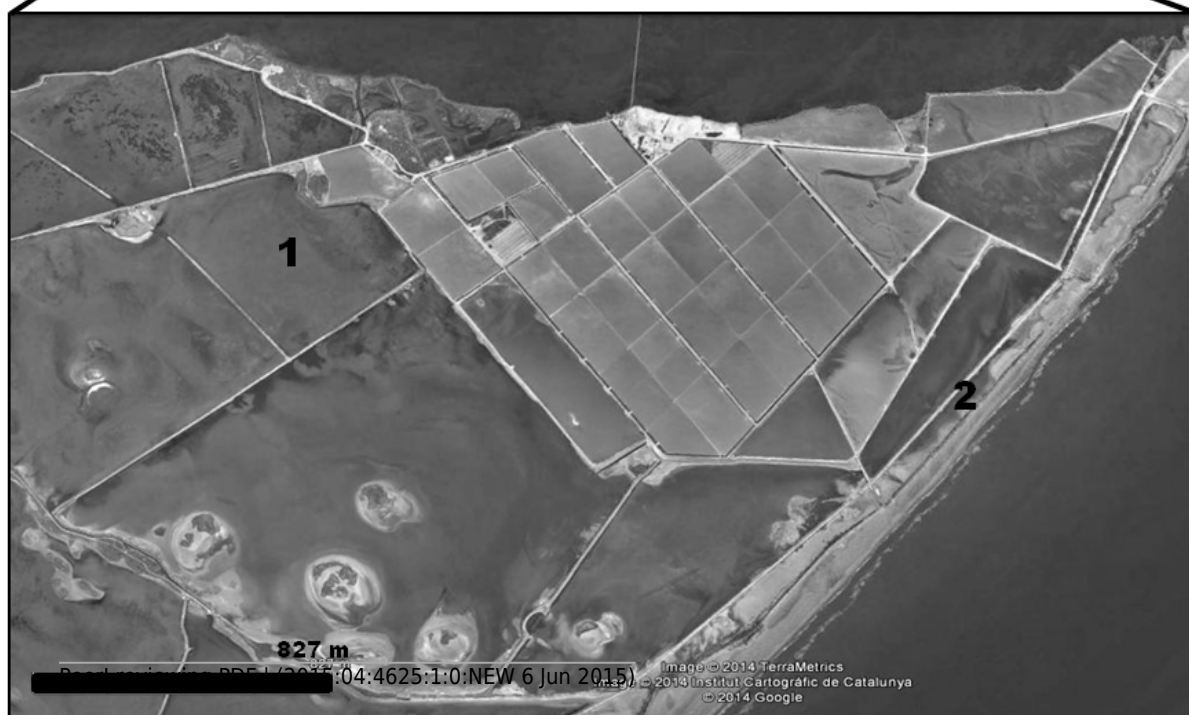
Mediterranean sea

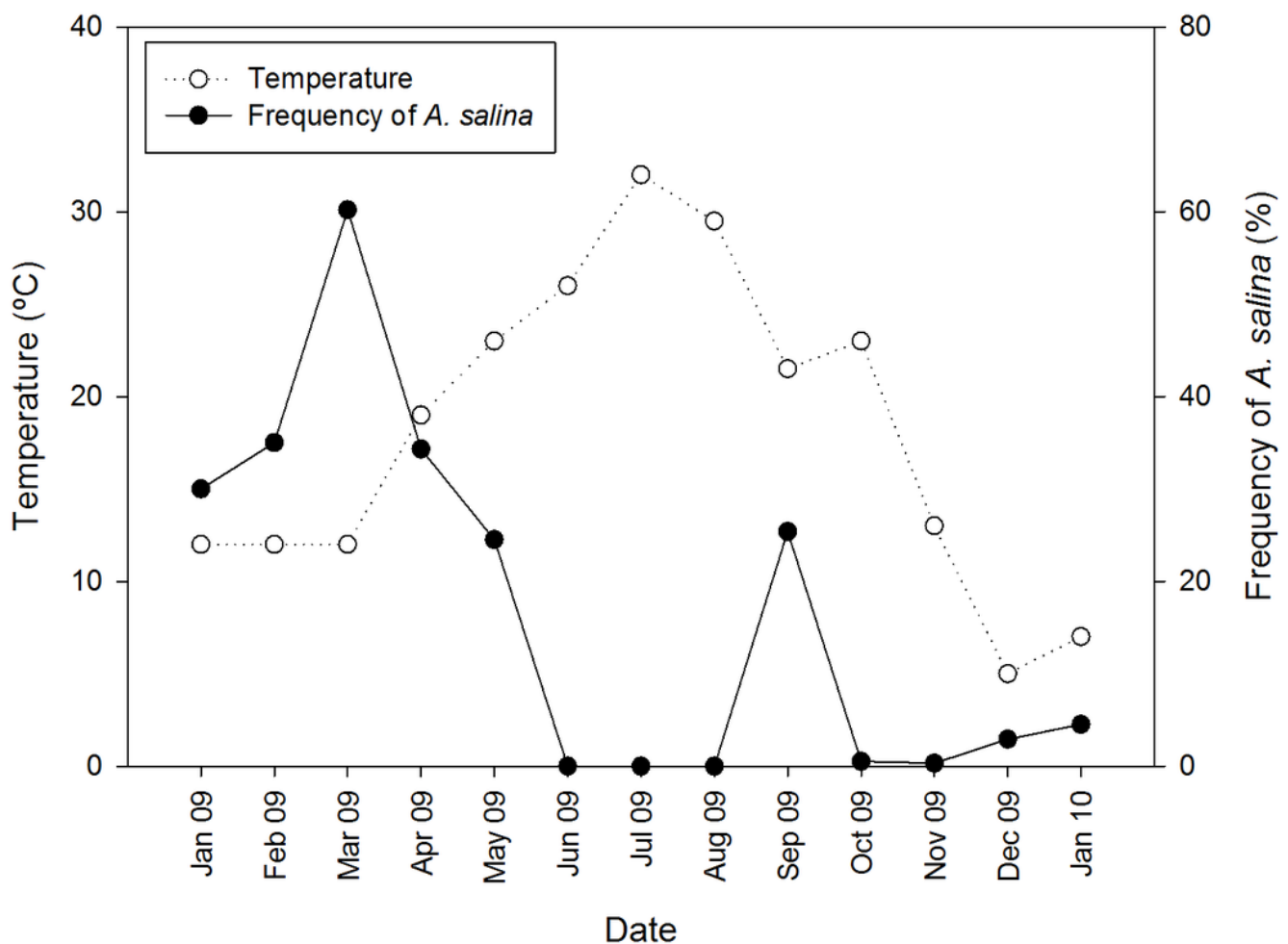
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image © 2015 Institut Cartogràfic de Catalunya

12.4 km

La Trinitat salterns

827 m

Image © 2014 TerraMetrics
Image © 2014 Institut Cartogràfic de Catalunya
©2014 Google

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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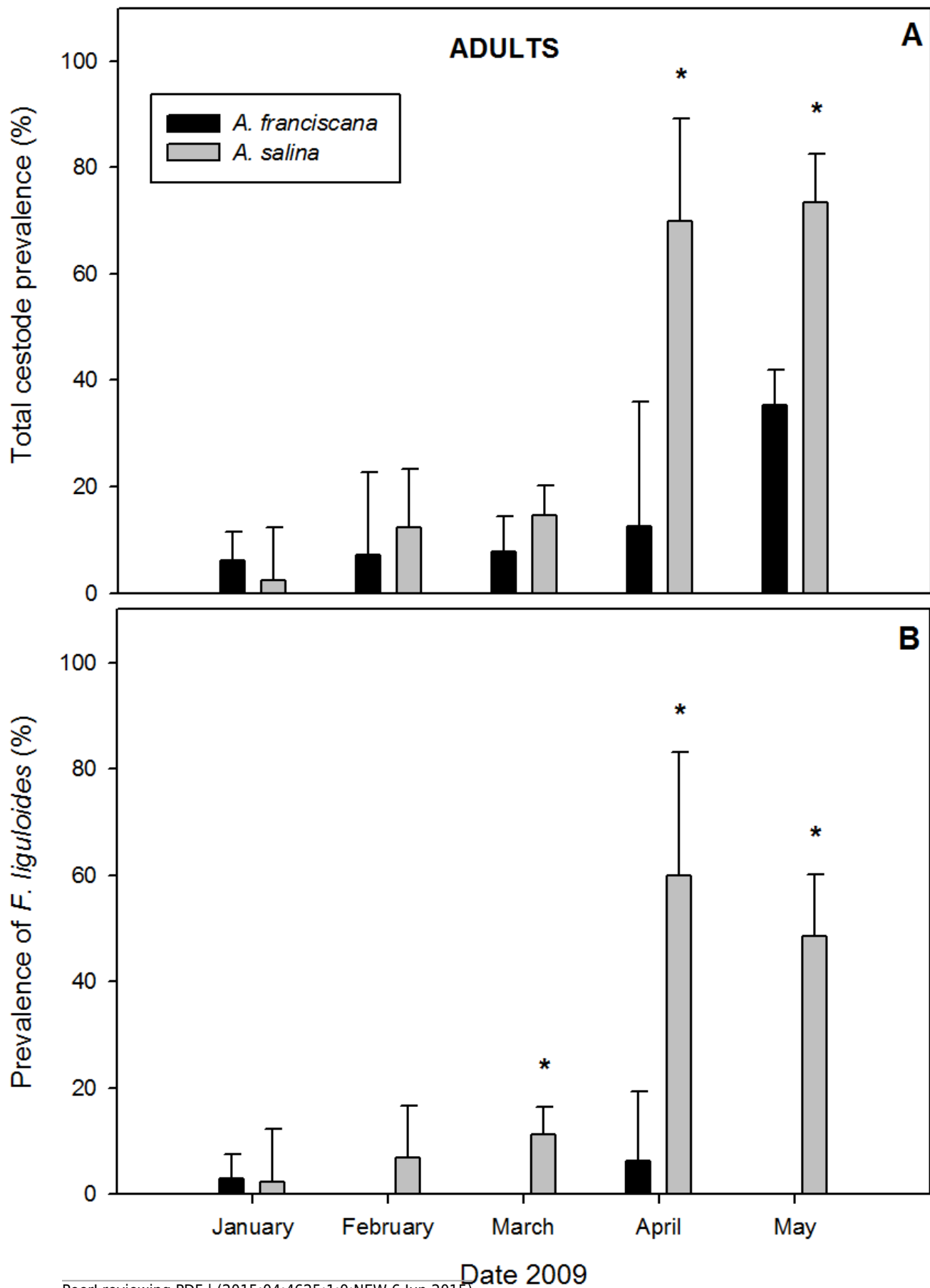
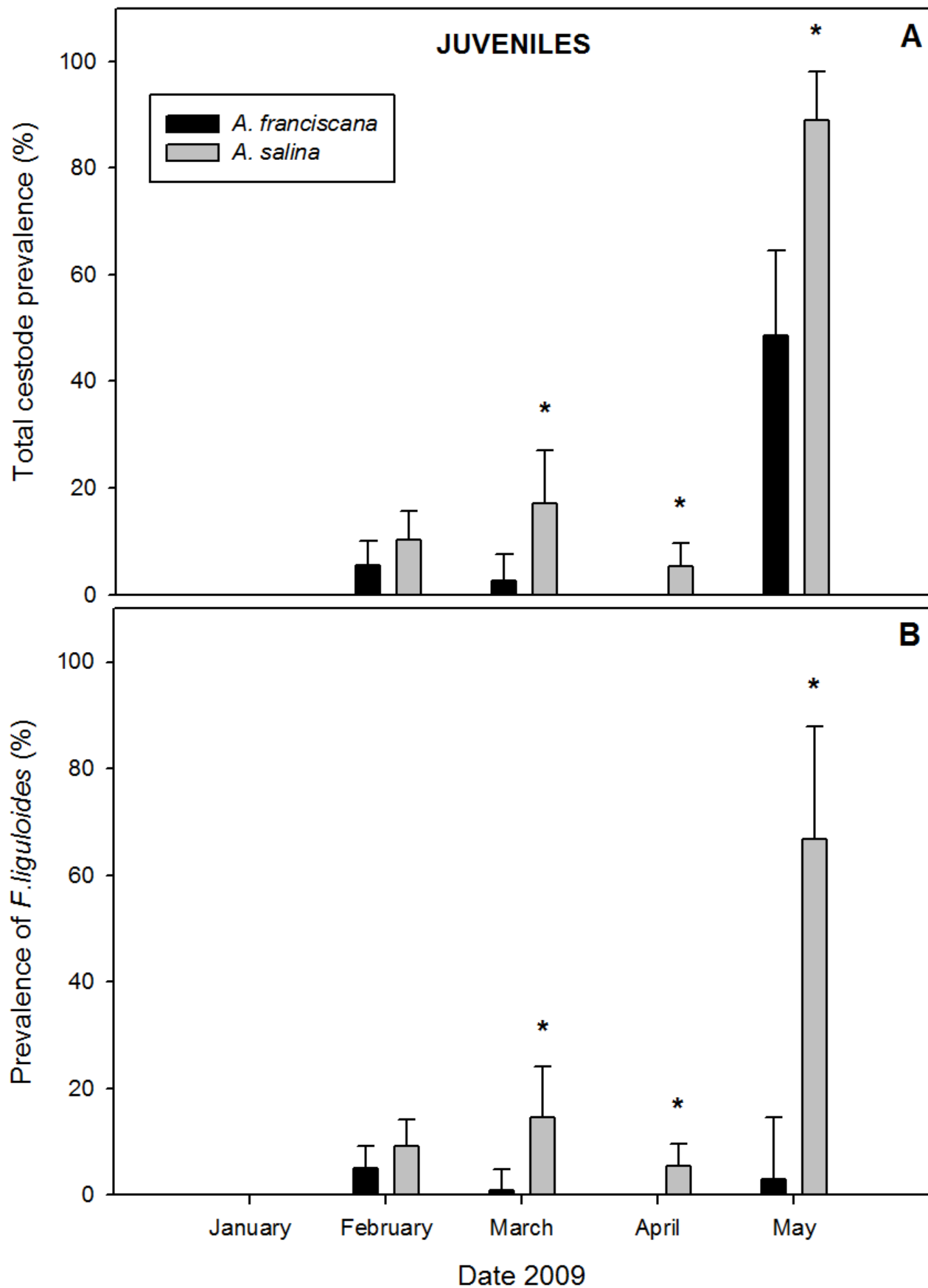
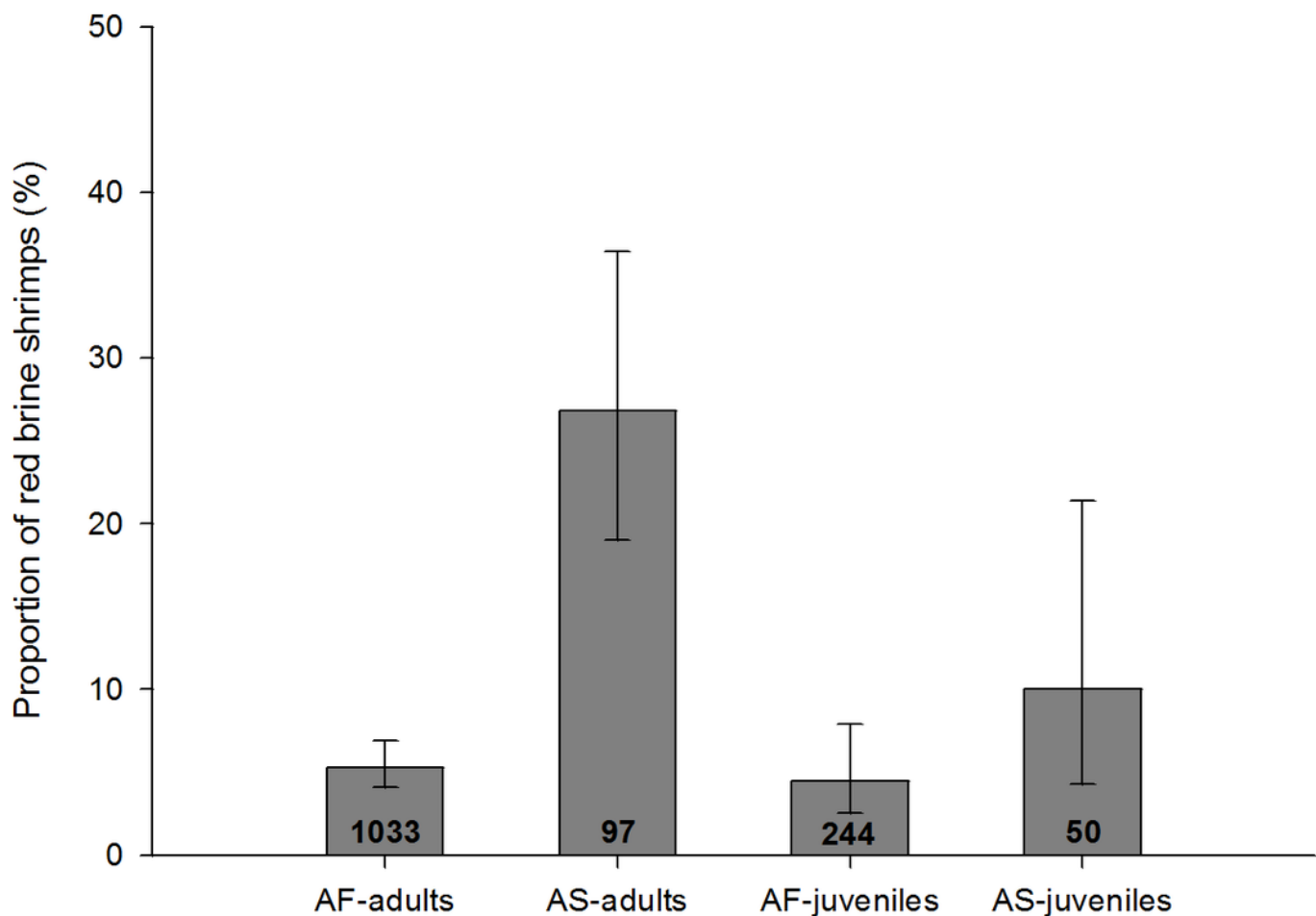
Comparative infection in adults of syntopic brine shrimp populations: *A. franciscana* and *A. salina* from pond CX, during months when they were co-existing.

(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.

Comparative infection in juveniles of syntopic brine shrimp populations: *A. franciscana* and *A. salina* from pond CX, during during months when they were co-existing.

(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.

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 uninfected *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 tests, $p < 0.001$).

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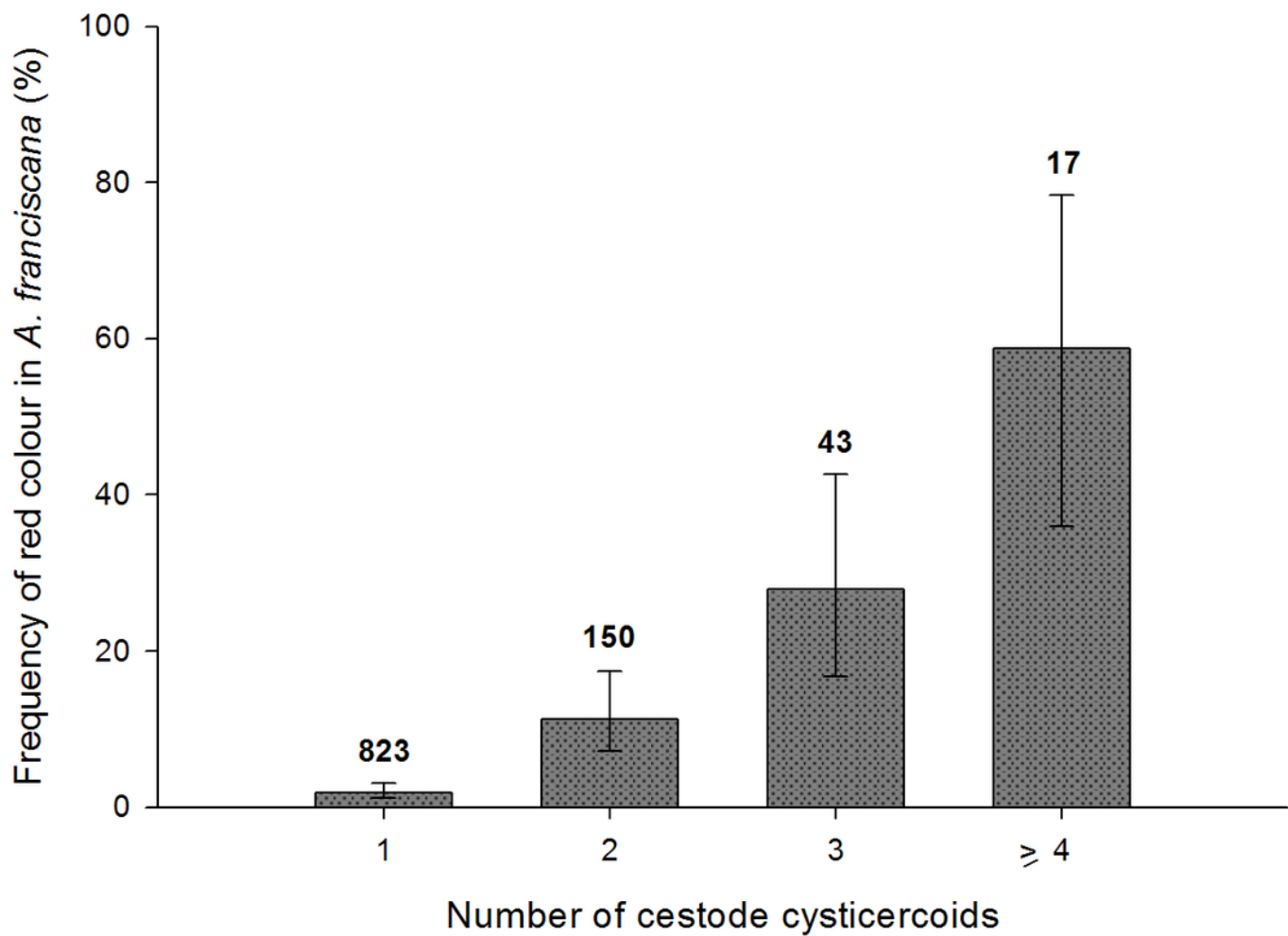
Colour pattern in relation to intensity of infection in adult *A. franciscana*.

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.