1 **Cestodes change the isotopic signature of brine shrimp *Artemia* hosts: implications for**
2 **aquatic food webs**
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28 **ABSTRACT**

29 To reach the final host (greater flamingos), the cestode *Flamingolepis liguloides* alters the
30 behaviour of its intermediate host, the brine shrimp *Artemia parthenogenetica*, causing them
31 to spend more time close to the water surface. In summer 2010 we showed that the prevalence
32 of this cestode was consistently higher at the top of the water column in the Odiel salt pans in
33 south-west Spain. We used stable nitrogen (N) and carbon (C) isotope analysis to test the
34 hypothesis that cestodes also alter resource use by *Artemia*. In early summer, we compared
35 stable isotopes in infected hosts at the surface with those from uninfected hosts at the bottom
36 of the water column. In late summer, we compared infected and uninfected *Artemia* from the
37 bottom. $\delta^{15}N$ was consistently enriched in infected individuals compared to uninfected hosts,
38 especially in *Artemia* with multiple infections of *F. liguloides* (family Hymenolepididae) and
39 those with mixed infections of *F. liguloides* and cestodes of the family Dilepididae. Infected
40 individuals from the surface were enriched in $\delta^{13}C$ compared to uninfected ones from the
41 bottom, but the opposite was found when comparing uninfected and infected *Artemia* from
42 the same depth. This may be caused by the increase in lipid concentration in infected *Artemia*.
43 Isolated cysticercoids of *F. liguloides* were significantly enriched in $\delta^{13}C$ compared to
44 infected hosts, but surprisingly were not enriched in N. Our findings illustrate the way
45 cestodes can alter food webs and highlight the importance of considering the parasitic status
46 of prey in studies of trophic ecology in saline wetlands.

47

48 *Keywords:* C and N stable isotopes, *Artemia*, cestodes, intermediate host, parasitism,
49 trophically transmitted parasite

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52 ## 1. Introduction

53 Parasites have often been ignored in aquatic food web studies (Lafferty et al. 2008). However
54 recent theoretical and empirical data suggest that parasites affect trophic interactions and
55 energy flow within ecosystems in a diversity of ways (Thompson et al. 2005; Lafferty et al.
56 2008). Trophically transmitted parasites, which depend on predation of their intermediate
57 hosts to complete their life cycle, have the potential to strongly impact trophic interactions
58 and food webs. Many parasites using trophic transmission have evolved sophisticated
59 mechanisms to modify the behaviour and morphology of their intermediate hosts to increase
60 the probability of predation by the final hosts (Moore 2002, Poulin 2007). However, the
61 ecological consequences of such manipulations at different trophic levels remain largely
62 unexplored.

63

64 *Artemia* spp. (Branchiopoda; Anostraca) are keystone species and dominant
65 macrozooplancton in hypersaline ecosystems (Wurtsbaugh and Gliwicz 2001) and can control
66 phytoplankton density via grazing (Lenz, 1987; Wurtsbaugh, 1992; Gliwicz et al. 2010).
67 Many species of aquatic birds depend on brine shrimps as a food source (Sánchez et al.
68 2007a, Varo et al. 2011, Vest & Conover 2011). Moreover *Artemia* is the intermediate host
69 for at least 15 species of cestodes which depend on predation by avian hosts (Georgiev et al.
70 2005, 2007). Brine shrimp become infected by ingesting cestode eggs released into the water
71 with the feces of the avian definitive host. The newly hatched oncosphere (tapeworm embryo)
72 penetrates into the hemocoel, where it develops into a cysticercoid (the larval stage). Birds
73 become infected via trophic transmission when they feed on infected *Artemia*. Adult
74 tapeworms develop and reproduce in the small intestine of birds, concluding their life cycle.
75 A particularly abundant cestode in brine shrimps is *Flamingolepis liguloides* (Cyclophyllidea,
76 Hymenolepididae), a parasite of flamingos. *F. liguloides* alters the size, fecundity, coloration,

77 physiology and behaviour of *Artemia* (Amat et al. 1991, Sánchez et al. 2006a, 2007b, 2009a,
78 Amarouayache et al. 2009). Previous studies show that behavioural changes (positive
79 phototactism) cause alterations in the spatial distribution of *Artemia*, with infected shrimps
80 being more abundant at the surface of the water and uninfected ones mainly occupying the
81 bottom of the water column (Gabrion et al., 1982; Thiéry et al., 1990; Sánchez et al. 2007b).
82

83 The aim of the present work is to explore potential trophic consequences of infection by
84 cestodes in *Artemia* using C and N stable isotope analysis (hereafter $\delta^{13}C$ and $\delta^{15}N$
85 respectively); $\delta^{15}N$ signature can inform about the trophic position, owing to enrichment
86 through the trophic levels; $\delta^{13}C$ signature provides information about the dietary source of
87 carbon. Stable isotopes have been increasingly used in studies of avian diet (Hobson and
88 Clark 1992a, b; Thompson and Furness 1995; Podlesak and McWilliams 2006, Gómez-Díaz
89 and Figuerola 2010) and constitute a powerful tool for studies of food webs (Post 2002).
90 However to date, studies of host-parasites relationships using C and N stable isotopes are still
91 scarce (Doi et al. 2008, Dubois et al. 2009, Gómez-Díaz and González-Solís 2010) and have
92 usually suggested that parasites have no effect on the isotopic signature of their hosts (but see
93 Miura et al. 2006).
94

95 In this paper we tested the following hypotheses: 1. Parasite-induced microhabitat segregation
96 affects $\delta^{15}N$ and $\delta^{13}C$ values of infected compared to uninfected *Artemia*. 2. The extent of this
97 modification depends on parasite load and taxa. We considered the effect of dilepidid
98 cestodes (Cyclophyllidea, Dilepididae) in coinfection with the dominant *F. liguloides*. Given
99 that both groups of parasites show different site preferences within the host's body likely to
100 reflect different feeding strategies (authors, unpublished), and may cause different
101 behavioural and physiological changes in their host, we tested whether $\delta^{13}C$ and $\delta^{15}N$

102 signatures were different between individuals infected with *F. liguloides* and those coinfected
103 with dilepidids. Owing to the dominance of *F. liguloides*, we were unable to study *Artemia*
104 infected only with dilepidids. 3. Isolated *F. liguloides* cysticercoids will be enriched in δ^{15}N
105 compared to hosts, because parasites are assumed to feed on host tissue.
106
107 **2. Material and methods**
108 *Artemia* sampling was conducted in a saltpan of the Odiel marshes (Huelva, SW Spain,
109 37°15'29"N, 6°58'25"W), a site of international importance for waterbirds (Sánchez et al.
110 2006b). The study area (1,118 ha) is a complex of ponds through which the sea water is
111 circulated and evaporated until salt precipitation. The Odiel brine shrimps are one of many
112 clonal populations in the Old World, often grouped under the binomen *A. parthenogenetica*
113 (Amat et al. 2005, Muñoz et al. 2010). Samples were collected in an evaporation pond of
114 moderate salinity (E18, salinity = 110-120g/l) during Summer 2010. This pond (labelled as I6
115 in Sánchez et al. 2006c) of constant depth (24-27cm) is a simple environment with no
116 structure from e.g. rocks or macrophytes (absent owing to the high salinity).
117
118 2.1 Distribution of infected and uninfected *Artemia* in the water column
119 In order to examine the proportion of infected and uninfected *Artemia* at the surface and depth
120 of the water column, we collected samples (between 0900 h and 1200 h) at 0-5cm from the
121 surface and 0-5 cm from the bottom during five different days from 30 June to 3 August 2010
122 at a fixed point 140 m from the shoreline. Samples were taken with an aquarium net (0.5 mm
123 mesh) at a constant, slow speed for 1 minute at depth (higher density) and 5 minutes at
124 surface. In the laboratory each individual was mounted in a temporary glycerol mount and
125 examined under a microscope for parasites (after Georgiev et al. 2005).
126

2.2. *Early summer sampling for isotope analysis: infected from the surface vs uninfected from the bottom*

In order to test if changes in the spatial distribution of *Artemia* induced by parasites had trophic consequences, we collected samples for isotope analysis in early-summer (5 occasions from 14 June to 5 July 2010 to reach an adequate sample size). No variations in food availability and biochemical composition of *Artemia* likely to affect isotopic composition were expected to occur during such a short sampling period. We collected infected *Artemia* from within 5 cm of the surface and uninfected ones from within 5 cm of the bottom with a 0.1 mm mesh plankton net. In the laboratory, brine shrimps were immediately placed in a tank filled with filtered (45 μm sieve) water from the same pond, during 24h to remove all food particles and to allow digestion of previously ingested food. Individual *Artemia* were then inspected under the stereomicroscope for the presence of parasites. More than 500 specimens where checked on each sampling date so as to reach a minimum of 475 individuals infected only with *F. liguloides* (mixed infection with other cestodes is very frequent, Georgiev et al. 2007). We always selected adult individuals of the same size range to minimize age variation (8-10mm, measured from the anterior margin of the head to the end of caudal furca).

Four categories were selected for isotope analysis on the basis of their abundance in the field: no parasites NP (n = 18 samples), infected with 1 *F. liguloides* (1FL, n = 7 samples), infected with more than 1 *F. liguloides* (>1FL, 2-14 cysticercoids per shrimp, n = 32 samples) and infected with both *F. liguloides* + Dilepidiae (FL+DIL 1-2 cysticercoids of *F. liguloides* and 1-2 cysticercoids of Dilepididae, n = 12 samples). Each sample was necessarily made from 10-20 individuals so as to provide enough material for isotope analysis. It was not practical to divide the *Artemia* with multiple infections of *F. liguloides* (between 2 and 14 cysticercoids) into more categories, owing to the very low number of individuals of intermediate categories

152 recorded, and the need to pool several individuals to achieve an acceptable weight for isotope
153 analysis (25 mg). We chose the categories of 1FL and >1FL because our unpublished data
154 suggest that host phenotype is affected by an increase from one to two *F. liguloides*
155 cysticercoids, but not by a further increase (e.g. red coloration becomes significantly more
156 intense with an increase between 1 and 2 cysticercoids, but not with a further increase).
157
158 In a field sample collected near the surface on 30 June and 5 July (N = 259) and quantified,
159 the proportions of *Artemia* in the four study categories were 29% NP, 18.1% 1FP, 32% >1FL
160 and 4.2% FL+DIL; 16.6% corresponded to other categories of infected shrimps (i.e. presence
161 of additional cestode species, and 74.4% of these individuals were coinfected with *F.*
162 *liguloides*). We were not able to distinguish between the three Dilepidae species infecting
163 *Artemia* (*Eurycestus avoceti*, *Anomotaenia tringae* and *Anomotaenia microphallos*) because
164 this would require dissection which would cause loss of most haemolymph. Cysticercoids of
165 *F. liguloides* are easily recognizable without dissection (Georgiev et al. 2005).
166
167 From samples taken on the same dates, we also isolated cysticercoids of *F. liguloides* from
168 the body of *Artemia* (n = 10 samples, each sample containing 2000 cysticercoids). These
169 cysticercoids were extracted from many samples of *Artemia* from the top of the water column,
170 which were then minced and sieved to extract the relatively hard cysticercoids. However,
171 hosts were not conserved after cysticercoid removal as most haemolymph was lost in the
172 dissection. Although a given sample may have contained multiple cysticercoids from the
173 same individual host, each host could not be represented in more than one sample, so
174 avoiding non-independence. The isotopic signatures of cysticercoids were compared to those
175 of intact uninfected and infected hosts. Cysticercoids of Dilepididae are much smaller and

176 show low prevalence in *Artemia* (Georgiev et al. 2005, 2007) so isolation of an adequate
177 quantity was not feasible.
178
179 *2.3 Late summer sampling for isotope analysis: infected from the bottom vs uninfected from*
180 *the bottom*
181 In late summer (8 September 2010) we collected an additional sample of *Artemia* from the
182 bottom in order to compare C and N ratios of infected and uninfected individuals at the same
183 depth. The aim was to test the consequences of parasitism for isotope signature irrespective of
184 those caused by microhabitat segregation. Host categories were similar to early summer
185 except that there were insufficient individuals of the FL+DIL category for analysis. Hence we
186 obtained NP (n = 25 samples, each made of 5 individuals), FL (n = 11 samples) and >1FL (n
187 = 10 samples). In the laboratory the samples were processed as described above for early
188 summer.
189
190 Prior to isotope analysis, no lipid extraction was carried out. Each sample was dried at 60ºC
191 for 24h and ground to a fine powder using a vibratory ball mill at 30 s^{-1} for 25s. See
192 "Supplementary Data S1" for detailed information on methods for isotope analysis.
193
194 2.4. *Statistical analysis*
195 We used Chi-square tests to compare the proportions of *Artemia* infected with *F. liguloides*
196 between the surface and the bottom of the water column (vertical distribution).
197
198 Comparisons of $\delta^{15}N$ between uninfected and different categories of infected *Artemia* were
199 performed with one-way ANOVA, followed by Tukey's HSD post-hoc tests. Kruskall Wallis
200 tests followed by Mann-Whitney U-tests were used to compare $\delta^{13}C$ between categories in

201 early summer when normality assumptions were not met, even after log and other data
202 transformations. Similarly, comparisons of isolated cysticercoids and *Artemia* were made with
203 t-tests for $\delta^{15}N$, and Mann-Whitney U-tests for $\delta^{13}C$. P values were always adjusted for
204 multiple comparisons via false discovery rate (FDR, Benjamini and Hochberg 1995). All
205 statistical analyses were conducted using Statistica 6.0 (StatSoft 2001).

206
207

208 **3. Results**

209 3.1. *Vertical distribution analysis*

210 The proportion of *Artemia* infected with *F. liguloides* was consistently greater at the surface
211 than below in all sampling dates (Figure 1). These differences were statistically significant in
212 three of the five dates (Figure 1).

213

214

215 3.2. *$\delta^{15}N$ analysis - early summer*

216 The range of $\delta^{15}N$ varied among the different groups studied (Table 1). We found statistically
217 significant differences in $\delta^{15}N$ between non infected *Artemia* (NP, n = 18; 7.76 ± 1.11‰,
218 mean ± SE), *F. liguloides*-infected *Artemia* (n = 39; 8.72 ± 0.06‰) and specimens infected
219 with both *F. liguloides* and Dilepidae (FLD, n = 12; 9.09 ± 0.15‰) (one way ANOVA: $F_{2, 66}$
220 = 41.90, P = 0.000001). Post-hoc Tukey's HSD tests (corrected for multiple testing) revealed
221 statistically significant differences between NP and *F. liguloides*-infected *Artemia* and
222 between NP and FLD (P < 0.001), but not between both categories of infected individuals.

223

224 When we repeated analyses differentiating between individuals with only one cysticercoid of
225 *F. liguloides* (1FL, n = 7) and those with more than one (>1FL, 2-14 cysticercoids, n = 32),
226 values of $\delta^{15}N$ also varied among groups (one-way ANOVA: $F_{3, 65}$ = 37.43, P = 0.000001).

227 $\delta^{15}N$ was significantly higher for >1FL (8.83 ± 0.054‰, mean ± SE) compared to those 1FL
228 (8.23 ± 0.167‰) (Tukey HSD, $P < 0.01$, Figure 2, Table 2). All other pairwise comparisons
229 were statistically significant, except between >1FL and FLD, and between NP and 1FL
230 (Figure 2A, Table 2).
231
232 The isotopic signature of isolated cysticercoids of *F. liguloides* ranged between 7.67 and
233 8.66‰ (Table 1). Cysticercoids were not significantly enriched in $\delta^{15}N$ compared to NP (8.00
234 ± 0.09 and 7.76 ± 0.11‰, mean ± SE respectively, t-test: $t = -1.52$, $P = 0.14$; Figure 2).
235 Cysticercoids were significantly depleted in $\delta^{15}N$ with respect to >1FL ($t = 7.58$, $P =$
236 0.000001) and FLD ($t = 6.062$, $P < 0.001$), but not 1FL ($t = 1.28$, $P = 0.22$, Figure 2A).
237
238 3.3. *$\delta^{13}C$ analysis - early summer*
239 $\delta^{13}C$ values were much more variable in all the groups than their respective N values (Table
240 1). The $\delta^{13}C$ values differed among NP (-26.08 ± 0.42‰, mean ± SE) and *F. liguloides*-
241 infected *Artemia* (-25.33 ± 0.22‰) and also FLD (-24.20 ± 0.45‰) (Kruskal Wallis test, $H (2,$
242 $N = 69) = 18.35$, $P = 0.0001$). Pairwise comparisons revealed statistically significant
243 differences between NP and *F. liguloides*-infected *Artemia* ($U = 167$, $P < 0.01$), between NP
244 and FLD ($U = 29$, $P < 0.001$) and between both categories of infected individuals ($U = 109$, P
245 < 0.01). Parasitized Artemia were enriched in $\delta^{13}C$ compared to NP (Figure 2B).
246
247 When we repeated analyses differentiating between 1FL and >1FL individuals, the average
248 values of $\delta^{13}C$ were almost identical between these two categories (-25.453 ± 0.63 for 1FL
249 and -25.303 ± 0.227‰ for >1FL, mean ± SE; $U = 92$, $P = 0.464$; Figure 2). All other pairwise
250 comparisons were statistically significant (Figure 2), except between 1FL and NP individuals
251 (-26.81 ± 0.424‰, mean ± SE; $U= 34$, $P = 0.079$; Table 2, Figure 2).

252 $\delta^{13}C$ values for isolated cysticercoids varied from –20.99 to −23.73‰. Cysticercoids were
253 significantly enriched in $\delta^{13}C$ with respect to NP (−23.256 ± 0.256‰ and –26.081 ± 0.424‰
254 respectively, mean ± SE, U = 167, P = 0.0016). Cysticercoids also showed higher values of
255 $\delta^{13}C$ compared to 1FL (−25.453 ± 0.637‰, U = 9, P = 0.011) and >1FL (−25.305 ± 0.227‰,
256 U = 36, P = 0.00025). However, there was no significant difference between cysticercoids and
257 FLD (U = 31, P = 0.06).
258

259 3.4. *$\delta^{15}N$ analysis - late summer*

260 The pattern of $\delta^{15}N$ for host groups in late summer (infected and uninfected from the bottom)
261 was equivalent to that observed in early summer (infected from the surface and uninfected
262 from the bottom). Different host groups varied in their range of $\delta^{15}N$ (Table 1), infected
263 individuals being enriched compared to NP. We found statistically significant differences in
264 $\delta^{15}N$ between NP (n = 25; 6.556 ± 0.047‰, mean ± SE), 1FL (n = 11; 7.115 ± 0.092‰) and
265 >1FL (n = 10; 7.322 ± 0.051‰; one-way ANOVA: F (2, 43) = 44.134, P = 0.000001). $\delta^{15}N$
266 was significantly higher for infected *Artemia* (both with multiple and simple infection)
267 compared to NP (Tukey HSD, P < 0.001; Figure 1A, Table 2) but not between 1FL and >1FL.
268

269 3.5. *$\delta^{13}C$ analysis - late summer*

270 Contrary to the pattern in early summer (when infected *Artemia* came from the surface)
271 individuals infected with >1FL showed the most negative $\delta^{13}C$ values (-27.438 ± 0.210‰,
272 mean ± SE), followed by 1FL (-26.982 ± 0.398‰) and NP (-26.462 ± 0.118‰, mean ± SE)
273 (Table 1, Figure 2B). The differences between categories were statistically significant (one-
274 way ANOVA: F (2, 43) = 5.261, P = 0.009). Pairwise comparisons revealed statistically
275 significant differences between NP and >1FL (Table 2).
276

277

278 ## 4. Discussion

279 The present study reveals strong differences in the isotopic signatures of C and N between
280 infected and uninfected *Artemia*. Using C isotopes, Miura et al. (2006) found that trematode
281 infection changed the habitat use and trophic ecology of marine gastropods, which moved to
282 the lower intertidal zone, where they were more susceptible to predation by their final hosts
283 (fish). However, other studies have reported no differences in stable isotopes of invertebrate
284 hosts when infected by trematodes (Doi et al 2008, Dubois et al 2009).

285

286 We interpret our results in a similar manner to Miura et al. (2006), and suggest that infection
287 by cestodes has direct trophic consequences for their brine shrimp host, at least partly because
288 the parasite induces movement towards the water surface. As expected from existing
289 literature, prevalence of infected *Artemia* (with *F. liguloides*) was higher at the surface of the
290 water. *A. parthenogenetica* undergo diel vertical migrations and typically spend most of the
291 daytime in the bottom 25% of the water column and most of the night in the other 75%
292 (Britton et al. 1986). Each individual does not stay at a fixed depth throughout the daily cycle,
293 and cestode infection changes the proportion of the time spent at different depths (Sánchez et
294 al. 2007b). The change in *Artemia* trophic ecology appears to be one of a suite of
295 interdependent host characters changed by infection, including colour, behavior and lipid
296 accumulation (Sánchez et al. 2006a, 2007b, 2009a). The differences found between infected
297 and uninfected shrimps collected together from the bottom of the water column are most
298 likely to be explained by the infected shrimps having spent more of their time at the top of the
299 water column than the neighbouring uninfected shrimps (and hence having consumed
300 different proportions of available food items), even though they were close together at the
301 moment of sampling.

302

303 An alternative explanation for the link between infection and isotopic signature would be that
304 it is an *effect* of inherent differences between individual shrimps in trophic ecology, not a
305 *cause*; i.e. that some individuals feed at a higher trophic level in such a way that they are more
306 likely to ingest cestode eggs and become infected. We cannot eliminate this possibility
307 without conducting experimental infections in the laboratory, but we consider it unlikely. In
308 any case, by comparing infected and uninfected shrimps from the same depth, we have shown
309 that infection status is a more important determinant of isotopic signature than position in the
310 water column at the time of sampling.

311

312 Stable isotope ratios of a parasite-host system can potentially shift because of the direct effect
313 of parasite biomass ('mass-balance' shift, Duboi et al. 2009). Changes in isotope ratios
314 between healthy and parasitized hepatopancreas of freshwater snails have been attributed to
315 the high proportion of trematode biomass (up to 3.4%) within this organ (Doi et al. 2008).
316 Given our inability to remove cysticercoids from *Artemia* without losing host fluids, we
317 estimated the contribution of the parasite to the total host biomass to evaluate whether the
318 presence of parasites have an important effect on the isotopic signature of *Artemia*. We
319 determined the average fresh weight of 60 infected *Artemia* within the size range used in our
320 study, and estimated the average mass of cysticercoids using the dimensions reported by
321 Georgiev et al (2005). Thus, we estimated that 1 cysticercoid would represent only $0.788 \times$
322 10^{-4}% of the total *Artemia* biomass. Individuals with multiple infections usually had 2 to 4
323 cysticercoids, with a maximum of 14. Thus, even when infestation load is highest, the
324 proportion of the host constituted by the parasite would not exceed 0.01%. Thus, the parasite
325 biomass makes a negligible contribution to the overall isotopic signature of the host.

326

327 Observed variations in N isotope ratios indicate that infected and uninfected *Artemia* may
328 occupy different trophic positions, as $\delta^{15}N$ increases at higher trophic levels (Bearhop et al
329 2004 ; Boecklen et al., 2011). According to $\delta^{13}C$ values, infected and uninfected individuals
330 used different carbon sources within the food web. We consistently found the strongest
331 differences between non-infected *Artemia* and those with either multiple or mixed infections,
332 suggesting a minimum number of parasites are required to make changes in stable isotope
333 signature most evident. This dose-dependent effect of parasites in their host is a common
334 phenomenon in nature in a variety of host-parasite associations (Blair and Webster 2007).
335
336 *Artemia* is considered a non-selective filter feeder (Provasoli and Shiraishi, 1959; Dobbeleir
337 et al., 1980), and is likely to feed on whatever microorganisms are present at the top or bottom
338 of the water column. At the salinity of our study pond, a diversity of autotrophic and
339 heterotrophic bacteria and eukaryotes are found in salt ponds, especially cyanobacteria, purple
340 bacteria, diatoms and protozoans (Pedrós-Alió et al. 2000, Estrada et al. 2004, Oren 2005).
341 These taxa are likely to vary greatly in their isotope signatures. *Artemia* are also known to
342 feed on detritus (Eardley, 1938; Conte and Conte, 1988; Wear et al., 1986, Savage and Knott
343 1998). Differences in $\delta^{15}N$ values between early and late summer indicate that, as well as the
344 influence of cestode infection on diet, there was also important seasonal variation in *Artemia*
345 diet likely to reflect a change in the relative abundance of different food items (Figure 2A). in
346 Higher $\delta^{15}N$ values in infected *Artemia* and in early summer may reflect a relatively greater
347 reliance on primary consumers (microzooplankton such as small ciliates and flagellates, and
348 heterotrophic bacteria) than on primary producers (phytoplankton such prokaryotic and
349 eukaryotic microalgae, and phototrophic bacteria). They may also reflect the consumption of
350 *Artemia* exoskeletons shed during moult which float at the water surface and are known to be

351 ingested by adult brine shrimps (Gliwicz et al. 2010), this being equivalent to cannibalism in
352 terms of isotopic enrichment.
353
354 The highly depleted values of $\delta^{13}C$ exhibited by non-infected individuals in early summer and
355 all *Artemia* in later summer (Figure 2B), from the bottom of the water column, suggest a diet
356 dependent on detritus and/or on benthic species that feed on detritus of terrestrial influence
357 (Fry and Sherr, 1984; Bouillon et al., 2002, Tavares et al. 2008). The detritus may originate
358 partly from halophytic Chenopodioideae that surrounds the study pond, since chenopod
359 detritus has similar depleted values (Cre´ach et al., 1997). In contrast, the higher C values for
360 infected individuals from the top of the water column suggest a marine contribution to the diet
361 (Fry and Sherr, 1984; Peterson et al., 1985), perhaps due to plankton and other C sources
362 entering with seawater pumped into the salt pond. Additional studies of gut contents of
363 infected and uninfected *Artemia*, e.g. by metagenomic analysis using New Generation
364 Sequencing (Boyer et al. 2012), are required to clarify the difference in diet between
365 parasitized and unparasitized individuals, and those at different depths.
366
367 A potential cause of shifts in the pattern of $\delta^{13}C$ for hosts from late summer (Figure 2B), with
368 infected individuals showing lower values than uninfected ones from the same depth is
369 parasite-induced host alteration of metabolic and physiological functions (pathological
370 effects). Modifications of host metabolism can involve changes in biochemical composition
371 of tissues or the whole animal (Thompson 1983 for review, Lauckner 1983, Plaistow et al.
372 2001), and different biochemical compounds have different stable isotope signatures (DeNiro
373 & Epstein 1977, Fantle et al. 1999, Pakhomov et al. 2004, Bodin et al. 2007). Changes in
374 isotope signatures between healthy and infected invertebrates caused by disturbances in host
375 metabolism have previously been reported (Dubois et al. 2009). In our study system, *F.*

376 *liguloides* increases the concentrations of lipids in *Artemia* (Amat et al 1991, Sánchez et al
377 2009a) which may cause shifts in isotope values since lipids are $\delta^{13}C$ depleted (DeNiro and
378 Epstein 1977; Lorrain et al. 2002; Bodin et al. 2007) and were not extracted from host tissues
379 prior to analysis. Reallocation of energy from reproduction to growth (due to parasite induced
380 host castration as reported in *F. liguloides*, Amat et al. 1991), may also contribute to our
381 results.
382
383 Cysticercoids of *F. liguloides* were significantly enriched in $\delta^{13}C$ relative to the host. This
384 result is consistent with the classical pattern that $\delta^{13}C$ increases slightly up the food chain
385 (DeNiro and Epstein 1978, 1981, see also Gómez-Díaz and González-Solís 2010). However
386 an unexpected result was that isolated cysticercoids of *F. liguloides* did not show significant
387 enrichment of $\delta^{15}N$ relative to their host, but rather the opposite trend. The consumer
388 (parasite) is usually expected to be enriched in N signatures with respect to its diet (host),
389 such enrichment typically being much higher than for C (Peterson and Fry 1987; DeNiro &
390 Epstein 1978, 1981, Hobson & Welch 1992, Post 2002; Checkley & Entzeroth 1985). For
391 endoparasites such as cestodes, there is typically a ^{15}N-enrichment from their hosts because
392 the parasites feed mainly on host tissues and fluids (Boag et al., 1998; Doucett et al., 1999;
393 Power and Klen, 2004; O'Grady and Dearing 2006). Nontheless, our results are not unique
394 (Lafferty et al 2008; Dubois 2009), and the lack of $\delta^{15}N$ enrichment in Greenland cod
395 cestodes relative to hosts was attributed to the trophic transfer of common dietary sources
396 (Power and Klein 2004). The inability of some endoparasites to synthesize amino acids and
397 the need to take them from their host (Deudero et al. 2002) has also been proposed to explain
398 slight or no $\delta^{15}N$ trophic enrichment of parasites (Dubois et al. 2009). No enrichment in N
399 may also be explained by the abbreviated metabolic pathways of parasites and the low rate of
400 excretion, tegument diffusion and respiration (Dubois et al. 2009). All these explanations may

401 apply to our system. Owing to the need to pool large numbers of cysticercoids from different
402 hosts to have enough sample for analysis, we do not know the exact infection types to which
403 these cysticercoids belonged (but see methods for the relative abundance of different infection
404 types). Hence, although they were significantly depleted compared to *Artemia* with multiple
405 and mixed infections, it remains unclear whether they were depleted compared to their hosts
406 on an individual basis.
407
408 We also found substantial differences in the level of enrichment of C and N among *Artemia*
409 infected with *F. liguloides* and those co-infected with metacestodes from three species of the
410 family Dilepididae. The isotope enrichment of *Artemia* with mixed infection (*F. liguloides* +
411 *Dilepididae*) compared to individuals infected with *F. liguloides* may be due to a dose-
412 dependent effect since a host with a mixed infection harbours at least two parasites.
413 Nevertheless, it is possible that cestodes from different families uses different resources in
414 their host, or alter host metabolism in a different way. While Dilepidid cysticercoids are
415 mainly found in the adult thorax, *F. liguloides* cysticercoids are predominantly located in the
416 abdomen of adult *Artemia* (authors' unpublished data, Amarouayache et al. 2009, Redón et al.
417 2010).
418
419 Changes in spatial distribution of *Artemia* induced by parasites appeared to change resource
420 consumption. Given the abundance of *Artemia* and their importance as food source for birds,
421 cestode parasites have the potential to alter food web properties, energy and mass flows in
422 hypersaline ecosystems. On the other hand, as major consumers of phytoplankton and organic
423 particulate matter in hypersaline waters, alterations of the diet in *Artemia* can potentially
424 cause strong cascading effects through alteration of grazing effects on phytoplankton and
425 bacterioplankton. Feeding by *Artemia* influences phytoplankton density and composition
426 (Mohebbi, F. 2010), although there is no previous information on the influence of parasites on

427 grazing. Higher grazing rates by infected individuals at the surface of the water may decrease
428 phytoplankton abundance, thus increasing water clarity (Lenz 1987, Wurtsbaugh 1992,
429 Gliwicz et al. 2010). Augmentation of light passing through the water column is likely to
430 affect the algal-cyanobacteria community at the bottom of the water column. For example, it
431 might favor phototrophic bacteria which are common beneath the cyanobacterial layers in
432 hypersaline microbial mats (DasSarma and Arora 2001). Laboratory grazing experiments with
433 infected and uninfected *Artemia* would provide further insight into the ecological implications
434 of parasite-induced changes in diet of *Artemia*.
435
436
437 This study shows that cestodes can induce strong changes in isotopic signatures of their
438 intermediate hosts, and that infected and uninfected invertebrates are not equivalent from a
439 trophic point of view. Parasitic status of invertebrates should be checked and incorporated
440 into aquatic food web research and studies of trophic ecology and diet of birds. Given that
441 stable isotopes can be used to assess the importance of specific prey in the diet of birds
442 (Alexander *et al.* 1996; Tavares et al 2009), this technique can evaluate the importance of
443 infected and uninfected prey as a food source for birds. On the other hand, in studies of avian
444 ecology, invertebrates are typically sampled as potential bird prey without considering the
445 presence of helminth parasites and their potential effects on isotopic signatures. This may be
446 an important source of error when determining the part that each invertebrate taxon plays in
447 avian diet. As a consequence of their manipulations of host colour and behaviour, birds are
448 often particularly likely to ingest invertebrates that have been parasitized (Sánchez et al.
449 2009b). The prevalence of parasites is likely to vary between those invertebrates ingested by
450 birds and those collected by biologists as potential food items, since the sampling methods
451 may be very different. Our work shows it is very important to take into account the parasite
452 communities in those taxa, and how they can influence the isotope ratios of avian prey.

453

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762 Table 1. Range of $\delta^{15}N$ and $\delta^{13}C$ (‰) in non infected *Artemia* (NP), *Artemia* infected with 1

763 *F. liguloides* (1FL), *Artemia* infected with more than one *F. liguloides* (>1FL), infected by

764 both *F. liguloides* and Dilepididae (FLD) and isolated cysticercoids of *F. liguloides* (Cy FL)

765 in early summer (June-July). n = number of pooled samples, each of which is made of 10-20

766 individuals.

767

	Early summer			**Late summer**		
	n	**$\delta^{15}N$ range (‰)**	**$\delta^{13}C$ range (‰)**	**n**	**$\delta^{15}N$ range (‰)**	**$\delta^{13}C$ range (‰)**
NP	18	(6.97, 8.32)	(-27.61, -21.79)	25	(6.07, 7.01)	(-27.72, -25.30)
1FL	7	(7.55, 8.65)	(-26.80, -21.80)	11	(6.81, 7.84)	(-29.01, -25.13)
>1FL	32	(8.26, 9.65)	(-26.65, -22.28)	10	(7.11, 7.54)	(-28.83, -26.68)
FLD	12	(8.41, 10.03)	(-27.61, -21.79)	…	…	…
Cy FL	10	(7.67, 8.66)	(-23.73, -20.99)	…	…	…

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784 Table 2. Results of pair-wise comparisons for $\delta^{15}N$ (Tukey HSD test) and $\delta^{13}C$ (Mann-
785 Whitney U test) between uninfected *Artemia* and different categories of infected individuals
786 (see Table 1 for details). P values are showed. *p< 0.05, **p < 0.01, ***p < 0.001 after
787 correction for multiple testing (FDR).

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		$\delta^{15}N$			$\delta^{13}C$		
		1FL	**>1FL**	**FLD**	**1FL**	**>1FL**	**FLD**
Early summer	**NP**	0.054	0.0002***	0.0002***	0.080	0.002**	0.0008***
	1FL		0.003**	0.0003***		0.464	0.018*
	>1FL			0.22			0.011*
Late summer	**NP**	0.0001***	0.0001***	…	0.207	0.009*	…
	1FL		0.1333	…		0.428	…

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FIGURE LEGENDS

Figure 1. Proportion of *Artemia* infected with *Flamingolepis liguloides* at the surface (dark) and below (light). Sample sizes are given above the bars. *p<0.01 after FDR correction.

Figure 2. Mean (± SE) values for A) δ^{15}N and B) δ^{13}C in early and late summer for non infected *Artemia* (NP), those infected with 1 cysticercoid of *F. liguloides* (1FL), infected with more than 1 cysticercoid of *F. liguloides* (>1FL), infected with both *F. liguloides* and Dilepididae (FL+DIL), and isolated cysticercoids of *F. liguloides* (C). Squares are samples from early summer, circles from late summer. Solid symbols are samples from the surface; empty symbols are samples from the bottom.

831 Fig. 1

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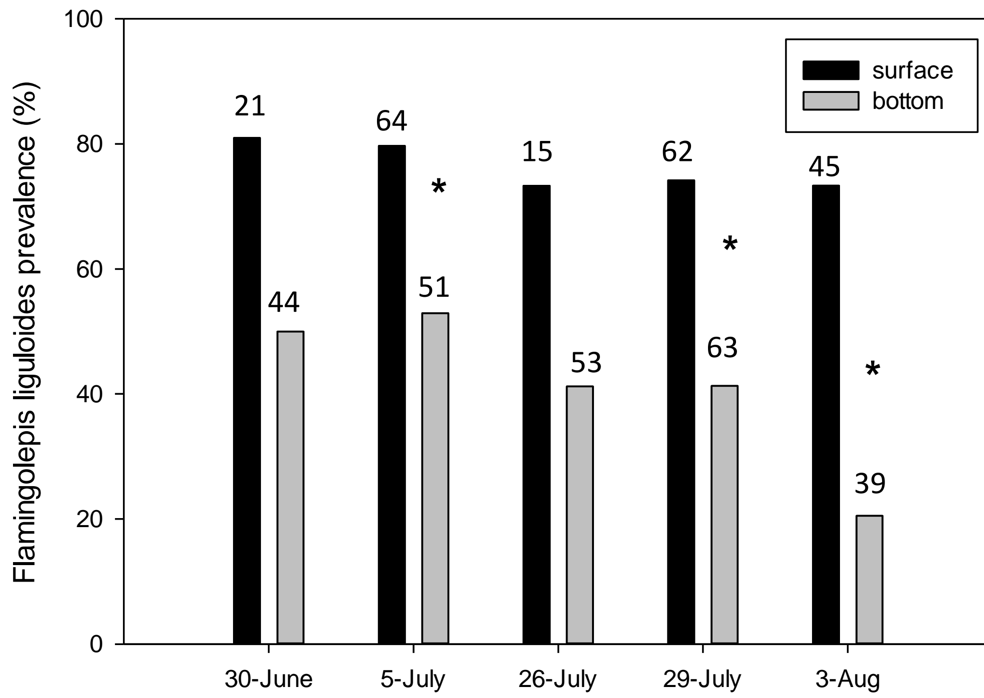

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858 Fig. 2

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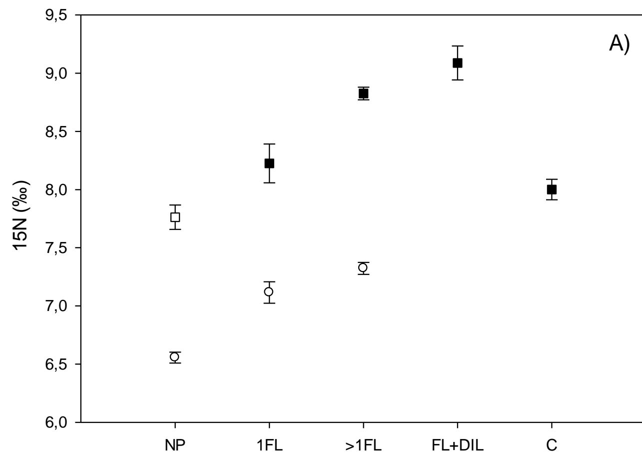

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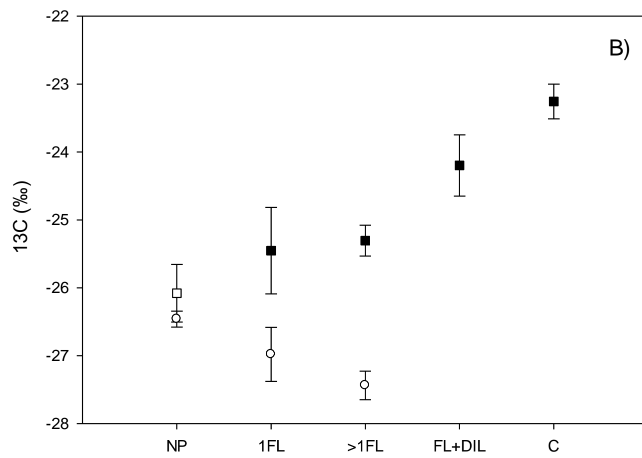

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875 **Supplementary material**

876 ## 1. $\delta^{15}N$ and $\delta^{13}C$ Analyses

877 Carbon and nitrogen isotope analysis was undertaken by EA-IRMS (elemental analyzer
878 isotope ratio mass spectrometry) by Isoanalytical laboratories (UK). In this technique,
879 samples and reference materials are weighed into tin capsules, sealed, and then loaded into an
880 automatic sampler on a Europa Scientific Roboprep-CN sample preparation module. From
881 there they were dropped into a furnace held at 1000 °C and combusted in the presence of
882 oxygen. The tin capsules flash combust, raising the temperature in the region of the sample to
883 ~ 1700 °C. The combusted gases are swept in a helium stream over a combustion catalyst
884 (Cr_2O_3), copper oxide wires (to oxidize hydrocarbons), and silver wool to remove sulphur and
885 halides. The resultant gases (N_2, NO_x, H_2O, O_2, and CO_2) are swept through a reduction stage
886 of pure copper wires held at 600 °C. This removes any oxygen and converts NO_x species to
887 N_2. A magnesium perchlorate chemical trap removes water. A Carbosorb trap is used to
888 remove CO_2 during nitrogen-15 analysis. Nitrogen or carbon dioxide is resolved by a packed
889 column gas chromatograph held at an isothermal temperature of 100 °C. The resultant
890 chromatographic peak enters the ion source of the Europa Scientific 20-20 IRMS where it is
891 ionised and accelerated. Gas species of different mass are separated in a magnetic field then
892 simultaneously measured on a Faraday cup universal collector array. For N_2, masses 28, 29,
893 and 30 are monitored and for CO_2, masses 44, 45, and 46 are monitored.

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895 The reference material used for $\delta^{15}N$ and $\delta^{13}C$ analysis was NBS-1577B (powdered bovine
896 liver, $\delta^{15}N_{Air}$ = 7.65 ‰, $\delta^{13}C_{V\text{-}PDB}$ = -21.60 ‰,). NBS-1577B, a mixture of IA-R045
897 (ammonium sulfate, $\delta^{15}N_{Air}$ = -4.71 ‰) and IA-R005 (beet sugar, $\delta^{13}C_{V\text{-}PDB}$ = -26.03 ‰) and
898 a mixture of IA-R046 (ammonium sulfate, $\delta^{15}N_{Air}$ = 22.04 ‰) and IA-R006 (cane sugar,
899 $\delta^{13}C_{V\text{-}PDB}$ = -11.64 ‰) were run as quality control check samples during sample analysis.

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901 NBS-1577B, IA-R045 and IA-R046 are calibrated against and traceable to IAEA-N-1

902 (ammonium sulfate, $\delta^{15}N_{Air}$ = 0.40 ‰). NBS-1577B, IA-R005 and IA-R006 are calibrated

903 against and traceable to IAEA-CH-6 (sucrose, $\delta^{13}C_{V\text{-}PDB}$ = -10.43 ‰). IAEA-CH-6 and

904 IAEA-N-1 are inter-laboratory comparison standards distributed by the International Atomic

905 Energy Agency (IAEA), Vienna.

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