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**Parasite-induced alteration of plastic response to predation threat: increased refuge use but lower food intake in *Gammarus pulex* infected with the acanotocephalan *Pomphorhynchus laevis*.**

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► **To cite this version:**

Lucile Dianne, Marie-Jeanne Perrot-Minnot, Alexandre Bauer, Arnaud Guvenatam, Thierry Rigaud. Parasite-induced alteration of plastic response to predation threat: increased refuge use but lower food intake in *Gammarus pulex* infected with the acanotocephalan *Pomphorhynchus laevis*. *International Journal for Parasitology*, 2014, 44 (3-4), pp.211-216. 10.1016/j.ijpara.2013.11.001 . hal-00957328

**HAL Id: hal-00957328**

**<https://hal.science/hal-00957328v1>**

Submitted on 6 Sep 2022

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1 Parasite-induced alteration of plastic response to predation threat:  
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4

5

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14

15 **Abstract**

16 Larvae of many trophically-transmitted parasites alter the behaviour of their intermediate host  
17 in ways that increase their probability of transmission to the next host in their life cycle.

18 Before reaching a stage that is infective to the next host, parasite larvae may develop through  
19 several larval stages in the intermediate host that are not infective to the definitive host. Early  
20 predation at these stages results in parasite death, and it has recently been shown that non-  
21 infective larvae of some helminths decrease such risk by enhancing the anti-predator defences  
22 of the host, including decreased activity and increased sheltering. However, these behavioural  
23 changes may divert infected hosts from an optimal balance between survival and foraging  
24 (either seeking food or a mate). In this study, this hypothesis was tested using the intermediate  
25 host of the acanthocephalan parasite *Pomphorhynchus laevis*, the freshwater amphipod  
26 *Gammarus pulex*. We compared activity, refuge use, food foraging and food intake of hosts  
27 experimentally infected with the non-infective stage (acanthella), with that of uninfected  
28 gammarids. Behavioural assays were conducted in four situations varying in predation risk  
29 and in food accessibility. Acanthella-infected amphipods showed an increase in refuge use  
30 and a general reduction in activity and food intake. There was no effect of parasite intensity  
31 on these traits. Uninfected individuals showed plastic responses to water-borne cues from fish  
32 by adjusting refuge use, activity and food intake. They also foraged more when the food was  
33 placed outside the refuge. At the intra-individual level, refuge use and food intake were  
34 positively correlated in infected gammarids only. Overall, our findings suggest that uninfected  
35 gammarids exhibit risk-sensitive behaviour including increased food intake under predation  
36 risk, whereas gammarids infected with the non-infective larvae of *P. laevis* exhibit a lower  
37 motivation to feed, irrespective of predation risk and food accessibility.

38 *Keywords:* Behavioural manipulation; Host protection; Refuge use; Foraging; Food  
39 intake; Risk-allocation; Acanthocephala; *Gammarus*

40

## 41 **1. Introduction**

42       Among parasites with complex life cycles and trophic transmission, many helminths  
43 enhance their transmission probability by altering the behaviour of the intermediate host in  
44 ways that increase its vulnerability to predation by definitive hosts (see Moore, 2002 for a  
45 review). This parasite strategy of host exploitation is thought to be the result of selection  
46 favouring parasite transmission (Lafferty, 1999; Chubb et al., 2010). However, because the  
47 earliest larval stages are not sufficiently well developed morphologically and physiologically  
48 to establish in the next host, any parasite-induced phenotypic change enhancing host  
49 susceptibility to predation at these stages would be counter-selected (Poulin, 1995). Several  
50 empirical studies have shown that such singular changes in host behaviour do indeed occur  
51 only when the parasite's larva is infective to the next host in the life cycle. Bethel and Holmes  
52 (1974) were the first to note that the acanthocephalan parasite *Polymorphus paradoxus*  
53 modifies the behaviour of its amphipod intermediate host (reversed phototaxis, skimming on  
54 the surface of water and clinging to floating objects) only when the parasite is infective to the  
55 bird definitive host. Similar observations were then made in the fish acanthocephalan parasite  
56 *Pomphorhynchus laevis* (Franceschi et al., 2008), as well as in cestodes (Poulin et al., 1992)  
57 and trematodes (McCarthy et al., 2000; Seppälä et al., 2005). Going one step further, Parker et  
58 al. (2009) predicted that, in addition to not favouring the predation of its host, a parasite  
59 variant able to protect its host against predation risks when non-infective should be selected  
60 relative to variants inducing no change in their hosts. To date, only two studies have  
61 experimentally supported this prediction (Dianne et al., 2011; Weinreich et al., 2013).

62       As suggested by Parker et al. (2009) and Dianne et al. (2011), such a parasite-induced  
63 protection of the host could divert host behaviour from an optimal strategy by impairing a  
64 balanced allocation to anti-predatory defences and foraging effort (seeking food or  
65 reproductive partners). Parasite-induced enhancement of anti-predatory behaviour is

66 particularly interesting to consider in the framework of the risk allocation hypothesis (Lima  
67 and Bednekoff, 1999). Under this hypothesis, prey are able to plastically modulate their  
68 foraging effort according to the magnitude and frequency of predation risk, in order to  
69 optimize energy intake. One can therefore wonder whether infection by a manipulative  
70 parasite impacts upon this plastic response. More specifically, parasites at a stage non-  
71 infective to the next host could increase anti-predatory defence by lowering the threshold  
72 response to predation risk, thereby decreasing food foraging. In such a case, infection by a  
73 non-infective stage would come at a cost to the host counterbalancing the benefit of increased  
74 protection from predators, overall decreasing host fitness and leading to host-parasite conflict  
75 (Schwartz and Koella, 2001). Alternatively, the parasite may affect the host's metabolism and  
76 energy needs in such a way as to decrease its motivation to feed, hence allowing a more  
77 intense use of refuge.

78 In this study, we tested the hypothesis of a trade-off between parasite-induced  
79 protection and food foraging in the freshwater amphipod *Gammarus pulex* infected with the  
80 acanthocephalan parasite *Pomphorhynchus laevis*. *Pomphorhynchus laevis* is a fish intestinal  
81 parasite at the adult stage. Contrary to uninfected *G. pulex*, amphipods parasitized by a *P.*  
82 *laevis* cystacanth (the infective larval stage) are photophilic (Cézilly et al., 2000; Franceschi et  
83 al., 2008), are attracted by predator odours (Kaldonski et al., 2007), show lower refuge usage  
84 (Kaldonski et al., 2007; Dianne et al., 2011), are less aggregative under predation risk  
85 (Durieux et al., 2012) and are frequently found drifting in the river (Lagrue et al., 2007).  
86 Conversely, acanthella-infected amphipods increase the use of refuge, thereby decreasing the  
87 risk of being preyed upon by a fish (Dianne et al., 2011). We hypothesized that the downside  
88 of high refuge use in acanthella-infected hosts is lower food foraging and food intake  
89 compared with uninfected amphipods. We measured amphipods' activity and levels of refuge  
90 use and food intake after experimental infection by acanthellae. These experiments were

91 carried out in two situations differing in the level of predation risk simulated by chemical cues  
92 from chub (fish). To further modulate the intensity of the trade-off between foraging and  
93 sheltering, there was additional manipulation of the level of foraging effort necessary to  
94 obtain food.

95  
96

## 97 **2. Materials and methods**

### 98 *2.1. Animals and experimental infections*

99 Uninfected *G. pulex* amphipods were collected in February 2012 from a small  
100 tributary of the Suzon River, Burgundy, eastern France (47°23'56.19"N, 4°50'31.13"E). Only  
101 male gammarids were kept for the experiment, since failure in parasite development is more  
102 often seen in female gammarids (Franceschi et al., 2008). They were acclimated in the  
103 laboratory for 8 to 10 days before experimental infections under a 12:12 light:dark cycle and  
104 fed during the experiment with conditioned elm leaves (*Ulmus laevis*). Adult *P. laevis*  
105 parasites were removed from the intestine of naturally infected chub (*Leuciscus cephalus*)  
106 caught in February 2012 in the Vouge River, France (47°09'34.36" N, 5°09'02.50"E) and  
107 characterized as described in Franceschi et al. (2008). Parasite eggs were collected from 12  
108 females of *P. laevis* sampled from four different fish.

109 Experimental infections were carried out following the procedure of Franceschi et al.  
110 (2008) and Dianne et al. (2011). Briefly, two male gammarids were allowed to feed for 48 h  
111 on a 1 cm<sup>2</sup> piece of elm leaf, on which 100 eggs were deposited. A total of 600 male *G. pulex*  
112 were exposed to parasite eggs. Two hundred control gammarids were handled and maintained  
113 under the same conditions as the exposed gammarids. After exposure, gammarids were  
114 maintained in groups of 15 individuals in a 0.5 L aquarium (16 x 10.5 x 7 cm) where water  
115 was automatically changed daily. From the sixth week after exposure, the presence of *P.*  
116 *laevis* acanthellae was checked once each week by inspecting gammarids using a dissecting

117 microscope. Acanthellae (translucent light-orange and shapeless larval stages) can be detected  
118 through the host cuticle. The infection procedure allowed acanthella to be obtained at the  
119 same age ( $10 \pm 1$  weeks post exposure). As soon as an acanthella was observed to be infected,  
120 control gammarids were isolated in a small dish (6 cm diameter) and deprived of food for 24 h  
121 prior to behavioural experiments.

122 To produce predator cues, 12 young chub, each 15 cm long, were kept in the  
123 laboratory at  $15 \pm 1^\circ\text{C}$  under a 12:12 light:dark cycle, in a tank filled with 80 L of tap water  
124 previously dechlorinated, oxygenated and UV-treated. Each chub was weighed and  
125 individuals were matched within a group so as to obtain a final concentration of fish cues  
126 corresponding to a biomass of  $3 \text{ mg.L}^{-1}$ . Chub were acclimated for 2 weeks prior to the  
127 experiments and fed with live gammarids in order to strengthen the predation signal  
128 (Wudkevich et al., 1997). The water from a similar tank, treated as previously described but  
129 without chub, was used as a control.

130

## 131 2.2. Activity assay

132 The activity of infected and control gammarids was recorded and analysed using a  
133 ViewPoint device and software (©Viewpoint Life Sciences, Inc. -2010, France). Gammarids  
134 were placed individually in Petri dishes (diameter 8.5 cm, height 1 cm) filled with either  
135 control water or scented water. After 2 min of acclimatization, gammarid activity was  
136 recorded with an infrared camera for 5 min (continuous recording), as the proportion of time  
137 spent swimming (movements at a speed above  $15 \text{ mm.s}^{-1}$ ) and distance covered. This speed  
138 threshold was determined based on preliminary tests, showing that below  $15 \text{ mm.s}^{-1}$   
139 discrimination is not always possible between gammarids moving at low speed (crawling) and  
140 inactive gammarids (still moving their pleopods for respiration) .

141

142 *2.3. Foraging and refuge use*

143 We manipulated the trade-off between sheltering and foraging for food by providing  
144 food either under or outside the refuge under two contrasting levels of predation threat.  
145 *Acanthella*-infected gammarids and control gammarids were introduced individually into a  
146 10.5 x 16 cm rectangular box filled with 330 mL of either control or scented water, with each  
147 gammarid kept in the same water type during measurements of both activity and foraging. An  
148 opaque refuge, consisting of half a terracotta saucer (8.5 diameter) in which a 1 cm<sup>2</sup> opening  
149 was made, was placed at one end of each aquarium, covering approximately 18% of the total  
150 aquarium area. At the opposite end of the aquarium, a wooden pick was fixed, on which food  
151 could be attached, in order to prevent the gammarid from bringing food back under the refuge.  
152 Food consisted of two pieces of conditioned elm leaves (diameter 1 cm<sup>2</sup> each) of known dry  
153 weight. Prior to each experiment, each leaf disc was dried for 2 h at 50°C, weighed to the  
154 nearest one hundredth of a milligram and then re-hydrated for 24 h before being used in the  
155 experiment.

156 Refuge use was recorded in each of four different situations: without or with predatory  
157 cues, and with food placed under or outside the refuge. After an acclimatization period of 30  
158 min, refuge use by gammarids was checked every 10 min over a period of 150 min. A score  
159 of 1 was given if the gammarid was inside the refuge and a score of 0 if it was found outside.  
160 For each gammarid, the total score of refuge use therefore ranged between 0 (gammarid  
161 always outside the refuge) and 15 (gammarid always inside the refuge). Measurements of  
162 refuge use began 1 h after recording general activity.

163

164 *2.4. Food intake measurement*

165 Following the recording of refuge use, gammarids were maintained in their box for 24  
166 h, after which the remaining leaves were dried and weighed (as described in Section 2.3), to



167 determine the amount of food eaten by each gammarid.

168 Gammarids were finally dissected to confirm their infection status. Parasites were  
169 removed and hosts were dried to determine their dry body mass to the nearest one hundredth  
170 of a milligram.

171  
172 *2.5. Statistical analyses*

173 The activity data were arcsin - square root transformed to meet conditions of normality  
174 and homoscedasticity. We then used a linear model to analyze the effects of infection status  
175 (infected versus uninfected), predation threat (presence versus absence of predator cues),  
176 gammarid dry weight and their second order interactions on gammarids activity.

177 Because data distribution of scores of refuge use never met conditions of  
178 homoscedasticity even after transformation and neither showed a Poisson distribution, only  
179 non-parametric analyses were possible, and interactions between factors were impossible to  
180 test. Data were therefore transformed to binomial values to perform a model including  
181 interactions between factors. We assigned a score of 1 to gammarids that were always scored  
182 in the refuge (total score of 15), and a score of 0 to gammarids that were scored at least once  
183 outside the refuge (total score between 0 and 14). A logistic regression was performed to  
184 analyze refuge use, with infection status, predation threat (without versus with), position of  
185 leaves (inside versus outside the refuge), gammarid dry weight and their second order  
186 interactions as potential explanatory factors. It is worth noting that this transformation fits  
187 well the distribution of score data (see results), and non-parametric analysis using  
188 untransformed data provided essentially the same results as this logistic regression for the  
189 main factors. To assess the effect of sizes, we used an Odds-Ratio index (OR) and its 95%  
190 confidence interval (CI) (Nakagawa and Cuthill, 2007). The OR ranges between 0 and  $+\infty$  and  
191 is significant when the 95% CI does not exceed 1.

192 The dry weight of leaves remaining at the end of the experiment was transformed

193 using a BoxCox transformation to comply with conditions of homoscedasticity. A linear  
194 model was used to analyse the effect of infection status, environment, leaf position, gammarid  
195 dry weight and their second order interactions on the amount of eaten leaves. We assessed  
196 effect sizes by using bootstrapped Cohen's  $d$  with bootstrapped 95% CI (Nakagawa and  
197 Cuthill, 2007). Cohen's  $d$  ranges between  $-\infty$  and  $+\infty$ , is significant when the 95% CI does not  
198 exceed 0, and the effect size is considered to be medium to strong if it exceeds 0.5.

199         The infection procedure did not allow for control of the parasite load. The infection  
200 intensity (number of parasites per infected gammarid) ranged from 1 to 11. Franceschi et al.  
201 (2008) and Dianne et al. (2012) showed that the number of parasites may influence a  
202 parasite's life history traits and notably the intensity of behavioural changes at the cystacanth  
203 stage. We therefore addressed the effect of parasite intensity at the acanthella stage. As in  
204 Franceschi et al. (2008) and Dianne et al. (2012), three categories of infected individuals were  
205 created: infected with one, two and more than two acanthellae ( $n = 68, 41$  and  $47$ ,  
206 respectively). The effects of these three categories of infection on refuge use and food  
207 consumption were then analysed using the same models as described above, but for infected  
208 individuals only, by replacing the factor "infection status" by a factor "infection intensity".

209         For each analysis above, we compared the Akaike Information Criterion (AIC) among  
210 all of the possible models (but always keeping the main effects in the models), and presented  
211 that one minimizing the AIC.

212         We ran Spearman correlations to analyse a potential link between gammarid activity  
213 and refuge use, and between refuge use and food consumption where foraging was made  
214 necessary by placing the food outside the refuge. These intra-individual associations between  
215 refuge use, activity and food intake were established without predatory cues (where higher  
216 activity and foraging are predicted), and with predator cues (where lower activity and  
217 foraging are predicted).

218 All analyses were performed with JMP 10.0 (© SAS Institute Inc., USA), except  
219 calculations of bootstrapped Cohen's d which were performed with R 2.11.1 (R Development  
220 Core Team).

221

222

### 223 **3. Results**

#### 224 *3.1. Activity*

225 Gammarids were less active when exposed to water-borne cues from chub compared  
226 with gammarids not exposed to predatory cues (Table 1, Fig. 1A). Acanthella-infected  
227 gammarids were significantly less active than uninfected gammarids, but this effect interacted  
228 with gammarids' dry weight: the positive relationship between weight and activity was only  
229 significant for infected individuals (Table 1, Fig. 1B).

230

#### 231 *3.2. Refuge use*

232 Acanthella-infected gammarids were more often found inside the refuge than  
233 uninfected ones, irrespective of other situations (Table 2). This significant difference is  
234 mainly due to the fact that uninfected hosts were 2.5 times more likely to use the refuge when  
235 food was inside than when food was outside (Table 2, Fig. 2, OR = 2.471 [1.238; 4.933]). By  
236 contrast, acanthella-infected gammarids did not adjust refuge use while accounting for the  
237 position of food (Fig. 2). Both acanthella-infected and uninfected gammarids used the refuge  
238 significantly more often when predator cues were present in the environment, wherever the  
239 food was positioned (Table 2, Fig. 2). The size of this effect was strong (OR = 0.147 [0.087;  
240 0.247]). Refuge use correlated with gammarid activity only in infected gammarids under no  
241 predation threat: the more active the gammarids, the less they used a refuge (Table 3).

242 Among infected gammarids, the number of acanthellae per host did not significantly

243 influence the use of refuge (Likelihood-Ratio  $\chi^2 = 0.0008$ , 2 d.f.,  $P = 0.99$ ), in a model also  
244 including predation threat (L-R  $\chi^2 = 28.29$ , 1 d.f.,  $P < 0.0001$ ), food position (L-R  $\chi^2 = 0.02$ ,  
245 1 d.f.,  $P = 0.89$ ) and gammarid dry weight (L-R  $\chi^2 = 0.005$ , 1 d.f.,  $P = 0.94$ ), and where none  
246 of the interactions were significant (not shown) (global model: L-R  $\chi^2 = 28.69$ , 5 d.f.,  $P <$   
247 0.0001).

### 248 249 3.3. Food intake

250 Acanthella-infected gammarids ate less leaf than uninfected ones, a difference due to  
251 two factors. First, uninfected gammarids ingested more food when leaves were inside the  
252 refuge compared with outside (Table 2, Fig. 3; effect size: Cohen's  $d = 0.501$  [0.162; 0.862]),  
253 a behaviour not found in acanthella-infected gammarids (Fig. 2A). Second, uninfected hosts  
254 responded to predation threat by eating more leaf compared with the situation without threat,  
255 a plastic response not observed in uninfected gammarids (Table 2, Fig. 3). The size effect of  
256 predation threat on food intake in uninfected gammarids was significant and moderate to  
257 strong ( $d = -0.51$  [-0.888; -0.155]). Finally, food intake was positively correlated with  
258 gammarid dry weight (Table 2); the larger the gammarids, the more food they ingested  
259 (whatever their infection status or the treatment).

260 Among infected gammarids, the number of acanthellae per host did not significantly  
261 influence the food intake, in a model also including predation threat, food position gammarid  
262 dry weight and their interactions (global model:  $F_{14,135} = 0.75$ ,  $P = 0.73$ ).

263 Refuge use correlated negatively with food intake only for infected gammarids under  
264 no predation threat (Table 3).

## 265 266 267 4. Discussion

268  
269 Our behavioural assays show that *G. pulex* amphipods infected by acanthellae of *P.*  
270 *laevis* increased their use of a refuge and decreased their activity and food intake compared

271 with uninfected amphipods. In addition, only refuge use was sensitive to water-borne cues  
272 from fish. By contrast, uninfected individuals exhibited risk-sensitive behaviour by increasing  
273 refuge use, decreasing activity and increasing food intake under predation threat. This  
274 suggests that the increased anti-predator defences induced by this parasite at the non-infective  
275 stage divert the infected hosts from an optimal balance between foraging and protection from  
276 predation.

277         In agreement with Dianne et al. (2011), we confirmed here that acanthella-infected  
278 gammarids displayed increased anti-predator defences: they were less active and used more  
279 refuge than uninfected hosts, whether or not water-borne predator cues were present. We  
280 found a weak correlation between these two traits only in infected animals not exposed to  
281 predator cues. The general difference in refuge use observed between acanthella-infected and  
282 uninfected gammarids was mostly due to lower anti-predator defences in uninfected  
283 gammarids when food was outside the refuge. Uninfected hosts were therefore able to adjust  
284 their behaviour, particularly the balance between foraging and defence against predators, to  
285 account for food accessibility and predation risk. Acanthella-infected gammarids did not show  
286 such context-dependent use of refuge.

287         Are there direct consequences of refuge use on food intake? Under conditions where  
288 the need to forage for food was enhanced (food provided outside the refuge), we found a  
289 negative correlation between refuge use and food consumption only in acanthella-infected  
290 gammarids. Parasite-induced correlation between two behaviours has already been observed  
291 in another host-parasite system involving amphipods (Coats et al., 2010). Stresses other than  
292 parasitism can also induce behavioural syndromes, a pattern that is thought to be adaptive  
293 (e.g. Bell and Sih, 2007 for a syndrome induced by predation risk). Here, however, if a  
294 reduced food intake was only the consequence of refuge use, food intake of acanthella-  
295 infected hosts should have been similar to that of uninfected hosts when leaves were placed

296 inside the refuge. However, acanthella-infected gammarids ingested less food than uninfected  
297 ones. A few studies have previously shown that *G. pulex* infected with *P. laevis* cystacanths  
298 did eat less than uninfected ones (McCahon et al., 1988; Brown and Pascoe, 1989). Other  
299 studies on acanthocephalan-infected crustaceans have reported either a decreased feeding rate  
300 (Hernandez and Sukhdeo, 2008; Medoc et al., 2011) or an increased feeding rate (Dick et al.,  
301 2010). More generally, the effect of infection on consumption of food by the host is  
302 widespread among parasites, both in helminths and protists (see Moore, 2002 for a review).  
303 However, here acanthella infection did not merely decrease food consumption. It rather  
304 prevented a plastic adjustment of food intake to both the food accessibility and the level of  
305 predation risk, as observed in uninfected gammarids. First, uninfected hosts ate more leaf  
306 inside the refuge than infected ones. Since refuge use was strong in both uninfected and  
307 infected animals when food was inside the refuge, it could not account for this differential rate  
308 in food intake. Therefore, the intensity of refuge use was not the only factor involved in food  
309 consumption. Second, uninfected gammarids ate more than infected ones when predator cues  
310 were present, but also ate more when predator cues were present compared with no predator  
311 cues. It should be noted that scores of refuge use were measured during the first 2 h of the  
312 experiment, when the intensity of predator cues was at its maximum, while food intake was  
313 measured over 24 h. Given that fish cues are known to decrease over time (Wisenden (2000)  
314 estimated that fathead minnows' cues could persist for 2-4 h following the removal of fish)),  
315 food intake probably lasted for a longer time than the cues' persistence. It could thus be  
316 hypothesised that food intake occurred after the peak of predator threat. Still, this result  
317 suggests that uninfected hosts were able to adjust their food consumption according to the  
318 level of predation risk, in accordance with the predation risk allocation hypothesis of Lima  
319 and Bednekoff (1999) (see also Ferrari et al. (2009) for a recent review). In many species,  
320 individuals are able to plastically adjust food intake in response to predation risk, by delaying

321 foraging until periods with lower predation risk and/or by increasing food intake per time unit  
322 during bouts of low predation risk. Here, the disappearance of predation cues with time  
323 (Wisenden, 2000; Bytheway et al., 2013) could mimic such a safer period after a burst of  
324 predation risk, during which food intake by uninfected gammarids increased. Increased food  
325 intake in uninfected gammarids exposed to predator cues could also result from increased  
326 energy needs following the stress of predation threat. Our experiment was not designed to test  
327 the risk allocation hypothesis directly. For this, gammarids should have been submitted to  
328 variations in the magnitude and frequency of predation risk during their maintenance (Ferrari  
329 et al., 2009). Here, prior to behavioural tests, gammarids were maintained in the laboratory  
330 under no other predation risk than cannibalism for 10 weeks. However, all individuals  
331 originated from a stream where predators occur, either occasionally such as brown trout  
332 (*Salmo trutta*), or more regularly such as salamander larvae (*Salamandra salamandra*)  
333 (personal observations). They therefore experienced predation risk prior to the experiment and  
334 it is not surprising to see that uninfected individuals were able to plastically adjust food  
335 consumption to the level of predation risk. By contrast, acanthella-infected hosts did not  
336 exhibit such a context-dependant behaviour. The number of parasites per host (infection  
337 intensity) did not modulate this loss of plastic response.

338         Our findings are also relevant to the putative evolution of parasite-induced behavioural  
339 manipulation. Under one evolutionary scenario, the behavioural changes induced by  
340 manipulative parasites have resulted from the exploitation of the host's compensatory  
341 responses (Lefèvre et al., 2008). Considering food intake, this hypothesis implies that the host  
342 infected by a protective parasite would be still able to plastically increase foraging and food  
343 intake to compensate for the energetic costs of infection. Such a plastic behaviour could be  
344 further exploited by parasites, because increased food intake would provide more resources  
345 for their own growth (Lefèvre et al., 2008). However, our results show that infection of *G.*

346 *pulex* with the acanthella stage of *P. laevis*, while increasing the anti-predator response,  
347 impairs optimal foraging and food intake. It is therefore unlikely that this behavioural  
348 modification, which is favourable to the parasite but not to host, results from an exploitation  
349 of the host's compensatory responses (Lefèvre et al., 2008). Alternatively, the protective effect  
350 of acanthellae could be a consequence of long-lasting physiological changes induced by this  
351 growing stage in its intermediate host, as tentatively suggested from the negative correlation  
352 between refuge use and food intake found in acanthella-infected gammarids only. Further  
353 studies are required to test this hypothesis

354

### 355 **Acknowledgements**

356 We thank Sébastien Motreuil and Aude Balourdet for their technical assistance in the  
357 field and at the laboratory. LD was funded by a joined doctoral grant from the CNRS, France  
358 and the Conseil Régional de Bourgogne, France. The study was funded by a research grant  
359 (Grant n°9201/AA/O/040/S00619) from the Conseil Régional de Bourgogne.

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440 **Figure captions**

441

442 Fig. 1. Effect of predator threat (A), gammarid dry weight (B) interacting with infection  
443 status, on gammarid activity. Empty symbols and dashed line are uninfected gammarids, solid  
444 symbols and solid lines are infected gammarids. In A, values are means  $\pm$  S.E.M. and sample  
445 sizes are given within bars.

446

447 Fig. 2. Refuge use by gammarids according to infection status (I, infected; U, uninfected), the  
448 position of food (inside or outside the refuge), and the absence or presence of water-borne  
449 predator cues. (A) Data expressed as the proportion of gammarids never scored out of the  
450 refuge during the whole experiment; (B) data expressed as the score of refuge use score (thick  
451 lines are medians, boxes show interquartile ranges and bars show interdecile ranges; a  
452 gammarid never scored in the refuge was given a score of 0 and a gammarid always scored in  
453 the refuge was given a score of 15). Numbers below the bars are sample sizes.

454

455 Fig. 3. Food intake, measured as the dry weight of eaten leaves (means  $\pm$  S.E.M.), in  
456 acanthella-infected and uninfected gammarids according to the position of leaves (inside or  
457 outside the refuge) and the presence of predator cues in the environment. Sample sizes are as  
458 in Fig. 2.

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462 Table 1. Analysis of variance testing the effects of infection status (acanthella-infected vs.  
463 uninfected hosts), Predation threat (presence or absence of water-borne predator cues),  
464 gammarid dry weight and their interactions on the activity of *G. pulex* (proportion of time  
465 spent swimming, after square-root arcsine transformation of data).  
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Source of variation	Num d.f. <sup>a</sup> ; Den d.f. <sup>b</sup>	F-ratio	P
Whole model	5 ; 279	6.487	<0.0001
Infection status	1; 279	4.505	0.035
Predation threat	1; 279	6.388	0.012
Gammarids dry weight	1; 279	10.327	0.001
Infection status * Predation threat	1; 279	3.592	0.059
Infection status * Gammarids dry weight	1; 279	4.752	0.030

471 <sup>a</sup> Numerator degrees of freedom.

472 <sup>b</sup> Denominator degrees of freedom.

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Table 2. Logistic regression (a) and Analysis of Variance (b) analyzing the effects of infection status (acanthella-infected vs. uninfected hosts), predation threat (presence or absence of predator cues), food position (inside or outside the refuge), gammarid dry weight and their interactions on (a) refuge use and (b) food intake, measured as the dry weight of eaten leaves.

Sources of variation	Likelihood ratio (in <i>a</i> ) or F-ratio (in <i>b</i> )	P
<i>(a) Refuge use, whole model</i>		
	69.557	<0.0001
Infection status	4.116	0.042
Predation threat	56.061	<0.0001
Food position	5.258	0.022
Gammarid dry weight	0.039	0.843
Infection status*Food position	4.401	0.036
<i>(b) Food intake, whole model</i>		
	6.840 (6, 278) <sup>c</sup>	<0.0001
Infection status	9.956 (1, 278)	0.002
Predation threat	5.571 (1, 278)	0.019
Food position	7.611 (1, 278)	0.007
Gammarids dry weight	4.585 (1, 278)	0.033
Infection status* Predation threat	10.659 (1, 278)	0.001
Infection status*Food position	4.162 (1, 278)	0.042

482 <sup>c</sup> Within parentheses: Numerator and denominator degrees of freedoms.  
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Table 3. Spearman correlations between activity and refuge use and refuge use and food intake, when food was provided outside the refuge.

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Series			Correlation Activity - refuge use		Correlation Refuge use - food intake	
Infection	Predator cues	N	$\rho$	P	$\rho$	P
U	absent	35	-0.157	0.368	0.162	0.352
U	present	37	0.038	0.824	-0.106	0.534
I	absent	38	-0.356	0.028	-0.395	0.014
I	present	39	-0.288	0.075	-0.278	0.086

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U : uninfected gammarids ; I : gammarids infected with acanthellae ; N: sample size.

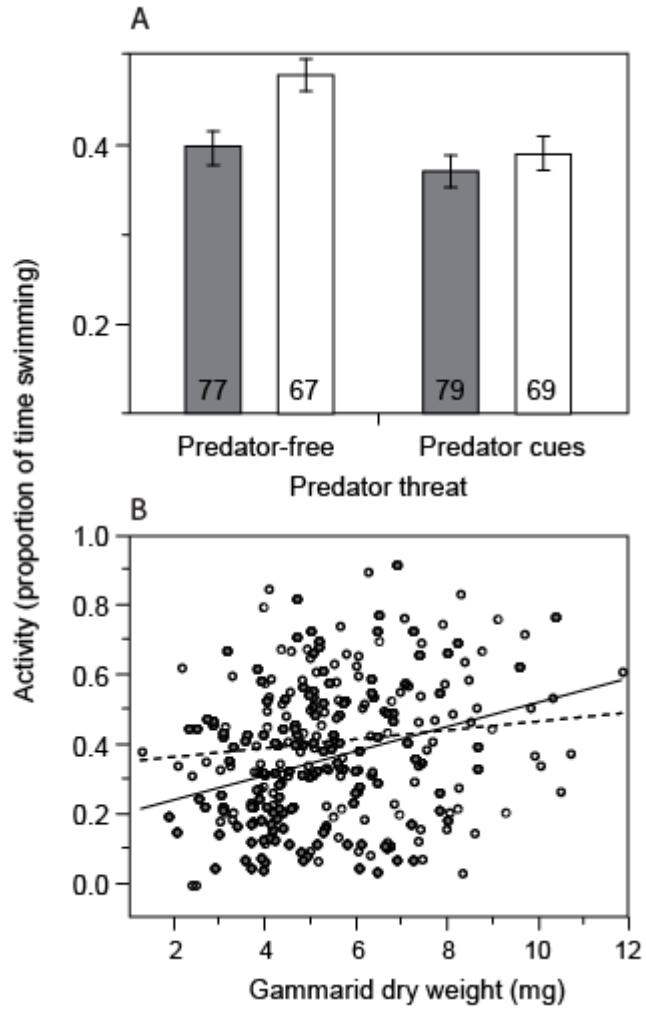
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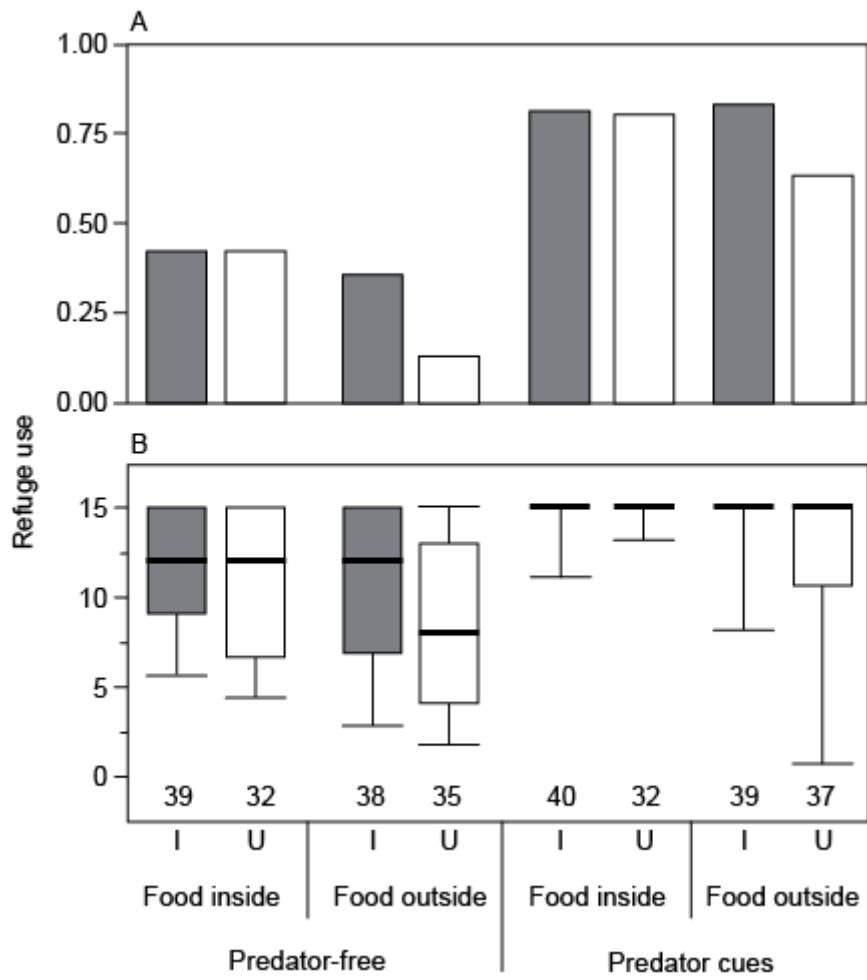
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Figure 1

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Figure 2

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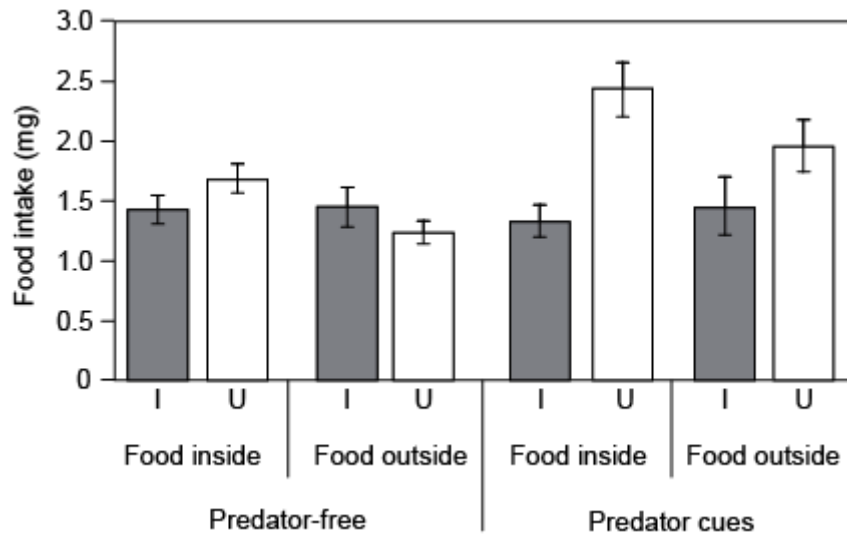
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Figure 3