

1 **Individual behavioral responses of an intermediate host to a manipulative**
2 **acanthocephalan parasite and effects of intra-specific parasitic competition**

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14 **Background:** Parasites with complex life cycles depend on the ingestion of their intermediate
15 host by the final host. To complete their life cycle successfully, parasites frequently
16 manipulate the intermediate host's behavior and appearance. Within host-parasite systems,
17 there is considerable variation in terms of intermediate hosts behavioral response to infection.

18 **Aim:** Identify sources of parasite-induced variation in intermediate host's traits by focusing
19 on intra- and inter-individual variation in behavioral responses to parasitic manipulation,
20 taking infection intensity, and thus, parasitic competition into account.

21 **Organism:** The acanthocephalan parasite *Polymorphus minutus*, which alters the phototactic
22 behavior and activity of its intermediate hosts, *Gammarus pulex*, thereby increasing the
23 probability to get eaten by final hosts.

24 **Methods:** We repeatedly examined the behavior of individual *G. pulex* varying in intensity of
25 infection with *P. minutus* from uninfected to multiple-infected. We analyzed phototactic
26 responses and activity.

27 **Results and conclusions:** Individual gammarids differed in phototactic behavior and in
28 activity patterns, with repeatability ranging from 20% to 50%. Infected gammarids showed
29 greater between-individual variation than uninfected gammarids in phototaxis but not in
30 activity. All uninfected gammarids were photophobic, whereas phototactic behavior of
31 infected gammarids ranged from photophobia to photophilia. On average, multiple-infected
32 gammarids were similarly photophobic as uninfected ones. Single-infected gammarids were
33 less photophobic than uninfected and multiple-infected conspecifics. This suggests that intra-
34 specific parasitic competition affects the manipulative abilities of parasites. Both groups of
35 infected gammarids were on average less active than uninfected ones, and this effect was
36 mainly driven by some infected individuals. In conclusion, behavioral variation of gammarids
37 was caused by individual differences in responses to manipulation/infection on the one hand,
38 and by the reduced manipulative capacities of parasites facing intra-specific competition on
39 the other hand.

40 **Introduction**

41 Parasites with a complex life cycle mature in an intermediate host species, but reproduce
42 sexually in a different, final host species (Schmid-Hempel, 2011). In order to achieve the host
43 change, it is often necessary that the intermediate host is ingested by the parasite's final host
44 (*trophic transmission*, Lafferty, 1999). This creates a strong selective pressure on the parasite
45 to increase the probability that its intermediate host is eaten by the final host (Moore, 2002).
46 While there are convincing examples that parasites manipulate the intermediate host's
47 behavior and appearance to successfully complete their life-cycle in some host-parasite
48 systems (Moore 1983; Poulin, 1999; 2010; Bakker *et al.*, 2017) there is still an ongoing
49 debate to which extent parasite-related changes in host phenotype increases transmission and
50 whether these changes are adaptive for the parasite (Cézilly *et al.*, 2010).

51 According to the *manipulation hypothesis*, parasites that are able to disturb or reverse
52 the anti-predator behavior or cryptic appearance of its intermediate host should benefit from
53 increased predation of the intermediate host (Moore, 2002). However, the evolutionary arms-
54 race between intermediate host and parasites needs not necessarily to be won always by the
55 individual parasite. This argument is supported by the occurrence of population-dependent,
56 differential manipulative abilities of parasites (Franceschi *et al.*, 2010a). Still, studies
57 examining individual behavioral variability of intermediate host are still underrepresented.
58 Instead, parasitic effects are usually examined using average values of behavioral or
59 morphological traits of infected and uninfected host individuals. Such approaches, however,
60 neglect within- and between-individual variation of host responses (Cézilly *et al.*, 2013;
61 Poulin, 2013). As selection requires phenotypic variation at individual level, detailed
62 knowledge about variance components and the factors maintaining variation are crucial in
63 order to gain a comprehensive understanding regarding the evolution of complex host-parasite
64 systems (Thomas *et al.*, 2011). Such variation in manipulative effects might depend on, for
65 instance, parasitic virulence (Alizon *et al.*, 2013), the intensity of infection and inter- as well

66 as intra-specific interactions between parasites (Mideo, 2009; Cézilly *et al.*, 2014), but also on
67 host resistance (Mazzi and Bakker 2003; Daoust *et al.*, 2015).

68 Acanthocephalans represent a well-described example of manipulative parasites
69 infecting arthropods as intermediate hosts and vertebrates as final hosts (Kennedy, 2006;
70 Bakker *et al.*, 2017). Infection with an acanthocephalan leads to altered appearance, behavior,
71 physiology and life-history of their intermediate hosts (see Bakker *et al.*, 2017 for a review).
72 Some of these changes are caused by active parasitic manipulation while others are adaptive
73 host responses to resist infection (Cézilly *et al.*, 2010; Bakker *et al.*, 2017). For example, the
74 acanthocephalan *Pomphorhynchus laevis* uses various *Gammarus* species as intermediate
75 hosts and certain fishes as final hosts (Kennedy, 2006). It alters the cryptic appearance of the
76 intermediate host as the conspicuous orange cystacanth (the infective developmental stage of
77 the parasite) is well visible through the cuticle of the gammarid (Kennedy *et al.*, 1978). Such
78 conspicuous color mark makes the intermediate host more prone to predation by three-spined
79 stickleback, *Gasterosteus aculeatus*, a suitable final host for *P. laevis* (Bakker *et al.*, 1997),
80 but not to *Salmo trutta*, representing an unsuitable host for *P. laevis* (Kaldonski *et al.*, 2009).
81 Furthermore, the parasite does not only change the intermediate host's visual appearance, but
82 also its anti-predator behavior. While uninfected *G. pulex* show predator avoidance and are
83 photophobic, individuals infected with *P. laevis* are attracted by predator odor (Baldauf *et al.*,
84 2007) and show photophilic behavior (Bakker *et al.*, 1997). These behavioral alterations are
85 assumed to increase the probability of predation of the intermediate host, and thus, the
86 transmission of the parasite to the final host (Lagrue *et al.*, 2007). The acanthocephalan
87 *Polymorphus minutus* exploits gammarids as intermediate and water birds as final hosts
88 (Kennedy, 2006). *Polymorphus* species alter the photo- and geotactic behavior of the
89 intermediate host, with infected amphipods being more photophilic and swimming closer to
90 the water surface (Hindsbo, 1972; Bethel and Holmes, 1974; Bailly *et al.*, 2017).
91 Furthermore, they reduce the overall activity of the intermediate host (Thünken *et al.*, 2010).

92 While such parasite-induced changes are well described on an average population
93 level, individual acanthocephalan-infected amphipods show considerable behavioral variation
94 (Thomas *et al.*, 2011), which can partly be ascribed to differential parasitic effects. For
95 example, modification of intermediate host`s behavior depend on the developmental stage of
96 the parasite. *P. laevis* and *P. minutus* are only infective at the cystacanth stage, but not at the
97 earlier acanthella stage (*P. laevis*: Franceschi *et al.*, 2008; Franceschi *et al.*, 2010b; *P.*
98 *minutus*: Bailly *et al.*, 2017). Consequently, parasites at different developmental stages have
99 different interests, which are reflected in their manipulative potential (Dianne *et al.*, 2010,
100 2011). While individuals that already reached the infective cystacanth stage shall try to
101 increase predation of the intermediate host by the final host, younger individuals in the
102 acanthella stage are expected to aim at avoiding predation (Hafer and Milinski 2015).
103 Furthermore, there are age-independent sources of manipulative variation. These include
104 season-dependent effects (Benesh *et al.*, 2009; Franceschi *et al.*, 2010b; Bailly *et al.*, 2017),
105 as well as genetic differences of individual parasites in the ability to manipulate the
106 intermediate host (Franceschi *et al.*, 2010a). Finally, the parasitization intensity, *i.e.* the
107 number of parasites within a single host, affects parasitic manipulation (Cézilly *et al.*, 2014).
108 In multiple-infected hosts, cumulated parasitic effects might result in increased manipulation
109 (Franceschi *et al.*, 2008). In contrast, competition between individual parasites over limited
110 host resources might impede parasitic growth and development (Cornet, 2011; Dianne *et al.*,
111 2012), resulting in reduced manipulation (Caddigan *et al.*, 2017), especially when
112 manipulation itself is costly (Maure *et al.*, 2013). Finally, parasites at different stages of their
113 life cycle might have opposite interests, which can lead to parasitic effects cancelling each
114 other (*sabotage hypothesis*, Haine *et al.*, 2005; Dianne *et al.*, 2010; Hafer and Milinski 2015).

115 Furthermore, differential responses to attempted manipulation by the parasite might be
116 caused by variation of the host individual itself. This variation might occur due to different
117 responses between host individuals or due to high within-individual behavioral inconsistency.

118 Infection may increase variation between hosts for example when certain individuals are
119 susceptible to infection whereas others are more resistant. Furthermore, infected individuals
120 may be less capable to maintain consistency in behavior, leading to higher within-individual
121 variation compared to uninfected individuals.

122 Thus far, these different sources of variation in intermediate host responses received
123 only limited attention, despite their importance to fully comprehend parasite-host-
124 coevolution. In the present study we i) describe within- and between-individual behavioral
125 variation in uninfected and infected *G. pulex* and ii) relate intensity of parasitic infection to
126 changes of host behavior. Therefore, we repeatedly tested photophobia and activity in
127 individual gammarids over a period of 17 days. Test animals were either uninfected or carried
128 at least one cystacanth of the manipulative parasite *P. minutus*. To test whether intra-specific
129 competition within a host affects parasitic manipulation, single-infected (no competition for
130 the parasite) or multiple-infected (competition between parasites) *G. pulex* were examined.
131 The competition hypothesis as well as the sabotage hypothesis predict weaker manipulation of
132 *Gammarus*. Alternatively, parasitic effects could add up and, thus, multiple-infected hosts
133 should suffer stronger from manipulation.

134 **Material and Methods**

135 **Experimental subjects**

136 Uninfected, single- and multiple-infected *Gammarus pulex* were collected on May 10th 2017
137 from the brook “Derlebach” in Bonn, Germany (50°42’N, 7°02’E). At the capture site, the
138 brook measured 50 cm in width and 15 cm in depth. The water temperature was 10°C. Several
139 hundred *G. pulex* were indiscriminately caught using a dip net and pre-sorted into uninfected
140 and infected individuals directly thereafter. Gammarids were transferred to the laboratory
141 using buckets filled with water and decaying leaves taken from the natural habitat. In the
142 laboratory, the infection status of the gammarids was determined visually by checking for the
143 presence and number of the orange cystacanths that were visible through the cuticle of the

144 dorsal coelom (Bakker *et al.*, 1997). Furthermore, gammarids were measured and dissected
145 directly after the experiments. Total length was defined as the distance between the base of
146 the first antenna and the base of the telson, measured to the nearest millimeter with the animal
147 placed on graph paper. Infected and uninfected gammarids did not significantly differ in size
148 (uninfected: 10.38 ± 1.89 mm, mean \pm SD; single-infected: 10.07 ± 1.32 mm, mean \pm SD;
149 multiple-infected: 9.38 ± 1.26 mm, mean \pm SD; Anova, $df = 2$, $F = 1.478$, $p = 0.242$). After
150 the experiment (see below) cystacanths were prepared out of all infected individuals. They
151 were photographed with tenfold magnification using a camera (Hitachi Denshi, HV-
152 C20AMP), attached to a stereomicroscope (Leica, S8AP0). Photos were used to verify
153 parasite species and infection status, i.e. number of parasites and developmental stage. All
154 parasites were cystacanths of *P. minutus*. Their proboscis was completely invaginated and the
155 parasites encased by an envelope and formed ovoidal (Dezfuli *et al.*, 2001). The number of
156 parasites in multiple-infected *G. pulex* varied between two to five (2.62 ± 0.26 , mean \pm SD).

157 In total 13 uninfected, 13 single- and 13 multiple-infected individuals were separated
158 and kept individually in plastic boxes (18.5 x 11.5 x 13.5 cm, length x width x height) filled
159 with 800 ml of aged tap water. Each box was equipped with an air stone and two gram of
160 decaying leaves, which served as food and shelter. Thus, individuals could choose between
161 bright (open area) and dark (under the leaves) light conditions. About 70 % of the water in
162 each box was replaced once a week with aged tap water. A full spectrum fluorescent tube
163 (True-Light, Natural Daylight 5500, 36W), emitting a spectral emission similar to natural
164 daylight, was placed in a distance of 41 cm above the holding boxes, creating a maximum
165 light intensity of 600 lux (PCE 174 Data logger light meter, PCE instruments). Gammarids
166 were kept at a light–dark cycle of 12L:12D and a temperature of 13 ± 1 °C.

167 Experimental design

168 Experiments were conducted between May 11th 2017 and May 27th 2017. Trials were
169 performed on three consecutive days (Tuesday to Thursday) each week, with all individuals

170 being tested once a day. Thus, each of the 39 gammarids was tested 9 times. For the
171 experiments, two clear plastic tanks, each measuring 24.5 x 15 x 15.5 cm (length x width x
172 height), were placed on a white Styrofoam plate, with the longer sides aligned to each other
173 (Fig. 1). Therefore, two trials could be conducted simultaneously. Tanks were filled with aged
174 tap water to a level of 7 cm. The water temperature of the experimental tanks resembled
175 holding conditions. The long sides of both tanks were covered with grey plastic sheets, so that
176 light could only reach the tank from the short end and from above. The set-up was illuminated
177 by a full spectrum fluorescent tube (True-Light, Natural Daylight 5500, 36W), installed at a
178 distance of 132 cm and at a height of 35 cm above water surface one short side of the set-up.
179 Thus, we created a brightness gradient within each tank (Figure 1). The light intensity in the
180 center of the light-facing half of the respective tank was 39 lux. In the center of the half turned
181 away from the light source light intensity was 31 lux. Above each tank we installed a webcam
182 (Logitech, Webcam Pro 9000) connected to a laptop (Fujitsu Siemens, Lifebook SH531). For
183 each trial, one gammarid was placed within a transparent plastic cylinder (diameter 3 cm) in
184 the middle of each tank. After an acclimation phase of one minute, the cylinders in both tanks
185 were lifted by hand, so that the gammarids were able to swim freely in their tank.
186 Immediately after lifting the cylinders, video recordings were started. A trial lasted 10
187 minutes. At the end of each trial, gammarids were carefully transferred back to their
188 respective holding boxes. To exclude potential side effects, the direction of the light source
189 was switched after every fifth trial.

190 Motion analyses

191 Video recording were analyzed using the tracking software Biobserve Viewer III (Biobserve
192 GmbH, version 3.0.0.119). The test tank was virtually divided into two equal sized zones, one
193 facing the light source (light) and the other one the opposing side (dark). The *Gammarus* was
194 continuously tracked for the experimental phase of 10 minutes. Time spent in each zone and
195 changes between zones were determined and exported to Microsoft Excel. A phototaxis index

196 was calculated (time on light side – time on dark side). Activity was estimated by the number
197 of changes between the light and dark side.

198 Statistical analyses

199 Statistical analyses were conducted in R, version 3.42 (R-Development-Core-Team, 2013).

200 When data deviated from normality they were Box-Cox-transformed or non-parametric test

201 were applied. Between-individual differences across and within infection groups (uninfected,

202 single-infected and multiple-infected) in phototaxis and activity were examined by fitting

203 linear models (lm) with individual gammarid as explanatory variable. To test for between-

204 individual behavioral variation among infections groups we first calculated mean values for

205 each gammarid and then compared variation among infections groups with Levene-tests. To

206 compare within-individual variation we first calculated a coefficient of variation (the ratio of

207 the standard deviation to the mean) for each gammarid and then compared infection groups

208 using Kruskal-Wallis rank sum tests. Repeatability was calculated with the R package "rptR"

209 (see Stoffel *et al.*, 2017). To examine behavioral differences among infection groups we

210 applied linear mixed effect models (lme using the R package "nlme") with activity or

211 phototaxis as dependent variables, infection group as explanatory factors and individual

212 gammarid as random factor. We added experimental day as covariates to the model to

213 examine whether phototaxis or activity changed over the course of the experiment and

214 whether this relationship differed between infection groups (day x infection group

215 interaction). Within infected gammarids, we investigated the effect of intensity of

216 parasitization (number of parasites within a host) on phototaxis and activity, respectively,

217 fitting linear models. The relationship between phototaxis and activity was examined using a

218 lm with phototaxis (based on mean value, see above) as response variable and activity as

219 explanatory variable. To test for differences in the relationship between phototaxis and

220 activity between infection groups we included the activity x infection group as interaction

221 term into the model. All non-significant interaction terms were removed from the models

222 (Engqvist 2005). All tests were two-tailed, alpha values smaller than 0.05 were considered as
223 being statistically significant.

224 **Results**

225 **Phototaxis**

226 Individual gammarids varied in phototaxis across infection groups (l_m, Δdf = 38, F = 8.498, p
227 < 0.001, Fig. 2a) as well as within groups (uninfected: l_m, Δdf = 12, F = 2.973, p = 0.001,
228 single-infected: Δdf = 12, F = 6.157, p < 0.001; multiple-infected: Δdf = 12, F = 8.503, p <
229 0.001). Infection status influenced phototaxis (Table 1). On average, single-infected
230 gammarids behaved randomly concerning phototaxis (one sample t-test, t = -0.723, Δdf = 12,
231 p = 0.483, Fig. 2b) and differed in phototaxis from multiple-infected gammarids and
232 uninfected ones (l_{me}, both Δdf = 1, both $\chi^2 > 3.897$, both p < 0.05, Fig. 2b). Multiple- and
233 uninfected gammarids did not differ significantly from each other (l_{me}, Δdf = 1, $\chi^2 = 2.744$, p
234 = 0.100, Fig. 2b) and both groups were on average photophobic (one sample t-tests, both Δdf
235 = 12, both t < -3.420, both p < 0.01, Fig. 2b). Individual variation in phototaxis differed
236 between infection groups (Levene-test, Δdf = 2, F = 4.142, p = 0.024, Fig. 2a), with single-
237 and multiple-infected gammarids being more variable than uninfected ones (Levene-tests,
238 both Δdf = 1, both F > 5.800, both p < 0.025, Fig. 2a). Single- and multiple-infected
239 gammarids did not differ significantly in this respect (Levene-test, Δdf = 1, F = 0.119, p =
240 0.732, Fig. 2a). All uninfected gammarids avoided the illuminated side, whereas in infected
241 gammarids, photophobic and photophilic individuals were present as well as those behaving
242 randomly concerning light response. During the course of the experiment (17 days),
243 phototaxis did not change significantly (Table 1). Within individual variation did not differ
244 significantly between infection groups (Kruskal-Wallis rank sum test, Δdf = 2, $\chi^2 = 0.560$, p =
245 0.755, see also table 2 for repeatability values). Within infected gammarids, number of
246 parasites did not significantly affect phototaxis (l_{me}, Δdf = 1, $\chi^2 = 0.020$, p = 0.886).

247 Activity
248 Individual gammarids varied in activity across infection groups (lm, $\Delta df = 38$, $F = 8.467$, $p <$
249 0.001 , Fig. 3a) as well as within each group (uninfected: lm, $\Delta df = 12$, $F = 3.072$, $p = 0.001$,
250 single-infected: $\Delta df = 12$, $F = 10.886$, $p < 0.001$; multiple-infected: $\Delta df = 12$, $F = 9.612$, $p <$
251 0.001). Infection status affected activity (Table 1). On average, infected gammarids (single-
252 and multiple-infected individuals did not differ significantly from each other (lme, $\Delta df = 2$, χ^2
253 $= 0.276$, $p = 0.599$), and were less active than uninfected individuals (lme, both $\Delta df = 2$, both
254 $\chi^2 > 5.517$, both $p < 0.02$, Fig. 3b). Between-individual variation did not significantly differ
255 between infected and uninfected gammarids (Levene-test, $\Delta df = 2$, $F = 1.124$, $p = 0.336$, Fig.
256 3a). Individual coefficient of variation did not differ significantly between infection groups
257 (Kruskal-Wallis rank sum test, $\Delta df = 2$, $\chi^2 = 0.560$, $p = 0.755$, see also table 2 for repeatability
258 values). In infected gammarids, number of parasites did not significantly affect host's activity
259 (lme, $\Delta df = 1$, $\chi^2 = 1.854$, $p = 0.173$).

260 Relationship between phototaxis and activity
261 Phototaxis did not significantly correlate with activity (lm, $\Delta df = 1$, $F < 0.001$, $p = 0.984$).
262 This effect was similar in infection groups (activity x infection group interaction: lm, $\Delta df = 1$,
263 $F = 0.361$, $p = 0.699$).

264

265 Discussion

266 Understanding individual behavioral variation is a classical topic in evolutionary and
267 behavioral ecology research (Bakker, 1986; Bell *et al.*, 2010) and has regained considerable
268 attention recently in the framework of animal personality (Barber and Dingemanse, 2010;
269 Beekmann and Jordan, 2017). However, individual variation in parasite-host interaction has
270 been considered to greater extent only recently (Thomas *et al.*, 2011; Poulin, 2013).

271 In our study, both uninfected and *P. minutus*-infected gammarids showed repeatable
272 individual differences in phototaxis and activity. Repeatabilities ranged from approximately

273 20% to 50% and were thus similar to those found in other behavioral traits in a range of
274 animal taxa (Bell *et al.*, 2010). Repeatability values for infected gammarids were higher than
275 those of uninfected ones. This probably resulted from higher between-individual variation in
276 infected gammarids compared to uninfected ones, as indicated by similar coefficients of
277 variation between infection groups. In line with these findings, Benesh *et al.*, (2008) found
278 repeatable activity in isopods infected with *Acanthocephalus lucii*, but not in uninfected ones.
279 In contrast, Coats *et al.* (2009) report higher repeatability in uninfected amphipods compared
280 to infected conspecifics. The contrasting results may reflect differences among species in
281 manipulative capabilities of parasites or host resistances (Franceschi *et al.*, 2010; Thomas *et*
282 *al.*, 2011, see below).

283 Phototaxis

284 Between-individual variation in phototaxis was greater between infected individuals. While
285 uninfected gammarids were uniformly photophobic (indicating strong selection on
286 photophobia), infected individuals showed the full behavioral range from photophobia to
287 photophilia.

288 The high variation observed in single-infected individuals might be explained by some
289 cystacanths having not yet reached the manipulative stage. Indeed, even at cystacanth stage it
290 takes further maturation or establishment within the host until manipulation becomes apparent
291 (Bethel and Holmes, 1974; Dianne *et al.*, 2010). Consequently, young cystacanths of *P.*
292 *minutus* and *P. laevis* are less manipulative than older ones (Franceschi *et al.*, 2008; Bailly *et*
293 *al.*, 2017). Bethel and Holmes (1974) showed that cystacanths of the closely related
294 *Polymorphus paradoxus* induce alterations in the host only 17 days after reaching that stage.
295 As we used naturally infected gammarids we do not have information about the exact age of
296 the parasite. However, if the described variation was caused by age effects one would expect
297 photophilia to increase over the course of the experiment in infected gammarids, as
298 cystacanths aged during this time as well. As we did not find any significant time effects, high

299 variation in manipulation most likely did not result from age differences between cystacanth.
300 Rather, it might either depend on the host's ability to resist manipulation, on individual
301 parasites manipulative abilities, or a combination of both. Indeed, it has been shown that
302 sibships of the manipulative acanthocephalan *P. laevis* differ in manipulative abilities (Cornet
303 *et al.*, 2009; Dianne *et al.*, 2012) and that gammarid hosts can develop resistance against local
304 manipulative parasites (Franceschi *et al.*, 2010a).

305 Interestingly, mean photophobic responses of multiple-infected gammarids were
306 comparable to those of their uninfected conspecifics. Thus, parasite's effects did not add up,
307 not leading to a stronger response. In contrast, our results suggest that intra-specific
308 competition among parasites dampens their manipulative effects. This effect can be explained
309 in two ways. First, intra-specific competition within the host might have affected the parasite
310 development (Dezfuli *et al.*, 2001; Franceschi *et al.*, 2008; Dianne *et al.*, 2010). Provided that
311 host resources are limited, and manipulation is costly, cystacanths sharing a host may need
312 longer to reach maximum manipulative potential. Second, parasites at different developmental
313 stages will have different manipulative interests. While older, highly infective cystacanths
314 aim at getting predated by a bird, younger ones would favor to remain in the intermediate host
315 for longer. Thus, lower photophilic behavior might be explained by cystacanths actively
316 competing over control of their *Gammarus* host.

317 Activity

318 In line with earlier studies (e.g. Thünken *et al.*, 2010) infected gammarids were less active
319 compared to their uninfected conspecifics. Interestingly, multiple-infected individuals showed
320 similar activity than single-infected gammarids and the number of parasites within a
321 gammarid was not significantly correlated with activity. This suggests that the additional load
322 by the parasite is not responsible for the changes in host's activity. Although infected
323 gammarids were on average less active, a proportion of infected individuals showed a similar
324 activity as uninfected ones (cf. Fig. 3a), suggesting that specific individuals only respond with

325 changes in activity or that reduced activity is only present at a specific time point, e.g. when
326 the parasite actively interferes with the physiology of the host. Furthermore, in contrast to
327 phototaxis, variation among individuals in activity was similar between infection groups,
328 supporting the findings of earlier studies that changes in activity are side-effects of the
329 infection rather than the result of active manipulation (e.g. Poulin, 1998; Thünken *et al.*,
330 2010). Future research should address these questions in more detail.

331 Another source of individual variation within infection groups might be the sex of the
332 gammarids. Indeed, acanthocephalan parasites reduce female fecundity (Bollache *et al.*,
333 2002). However, evidence for sex-specific behavioral responses to infection is ambiguous.
334 Park and Sparkes (2017) found that *Acanthocephalus dirus*-infected males and females of
335 *Caecidotea intermedius* differ in refuge use while Bailly *et al.* (2017) did not find sex-specific
336 phototactic responses of *P. minutus*-infected gammarids. We did not explicitly determine the
337 sex of the gammarids used in our study. However, animals of the different infection groups
338 were similar in size. Given the size-range of the used animals suggests that we used both male
339 and female *G. pulex* (Adams and Greenwood, 1983). Therefore, the differences between the
340 three different groups cannot be explained by sex differences. However, the variability within
341 the infected groups might be caused by different reactions of infected males and females. This
342 hypothesis might be investigated in more detail in future studies.

343 In summary, we show that individual gammarids differ in their risk-adverse behavior.
344 Furthermore, we demonstrate high variation in manipulative success of an acanthocephalan
345 parasite, which could be explained by between-parasite competition within an intermediate
346 host and differential responses of individual hosts to manipulation. Such variation in
347 responsiveness underlines the ongoing arms-race between the parasite and its host and shed
348 light on the evolution of trophical transmitted parasites and their hosts.

349 **Author contributions**

350 TT, SV, and SAB conceived the study. SV and TJ conducted the experiments. TT analyzed
351 the data. TT, SV and JGF discussed the results. TT, SV and JGF wrote the manuscript. The
352 final draft was approved by all authors.

353 **Acknowledgement**

354 This article is dedicated to Prof. Theo C. M. Bakker on the occasion of his retirement as a full
355 professor in Behavioral Ecology. We are greatly indebted for his invaluable continuous
356 support during all stages of our scientific careers. We thank Ingolf Rick and Marion Mehlis
357 for discussion and 2 anonymous reviewers for valuable comments. We thank Prof. Thomas
358 Bartolomaeus for logistic support. TT and SV were supported by a DFG grant (TH 1615/3-1)
359 to TT.

360

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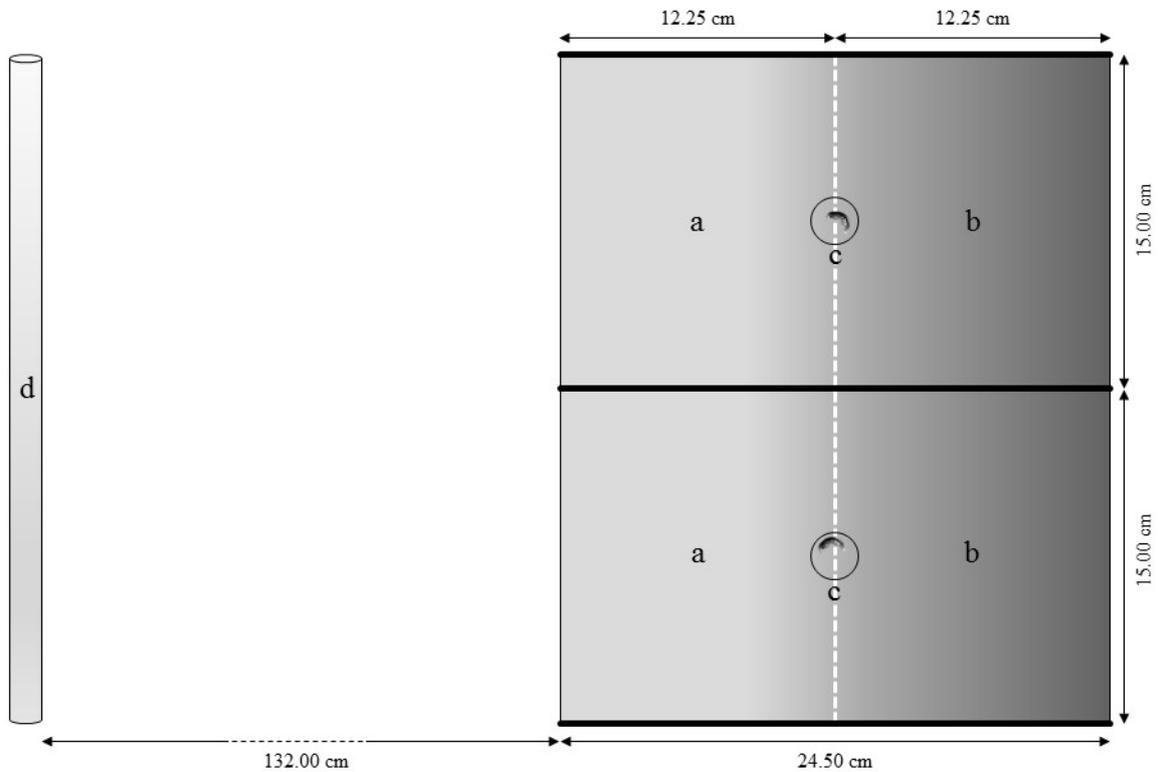
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- 493

494 **Figures:**

495 Figure 1

496



497

498 Fig. 1: Schematic figure of the experimental set-up. Two plastic tanks were placed alongside

499 to each other and visually separated. A brightness gradient was created by placing a slightly

500 elevated light source (d) 132 cm away from one side of the set-up. For tracking-software

501 analyses, two virtual zones were created with one facing the light source (a) and an opposing

502 one (b). The transparent cylinder (c) was lifted after one-minute acclimation time.

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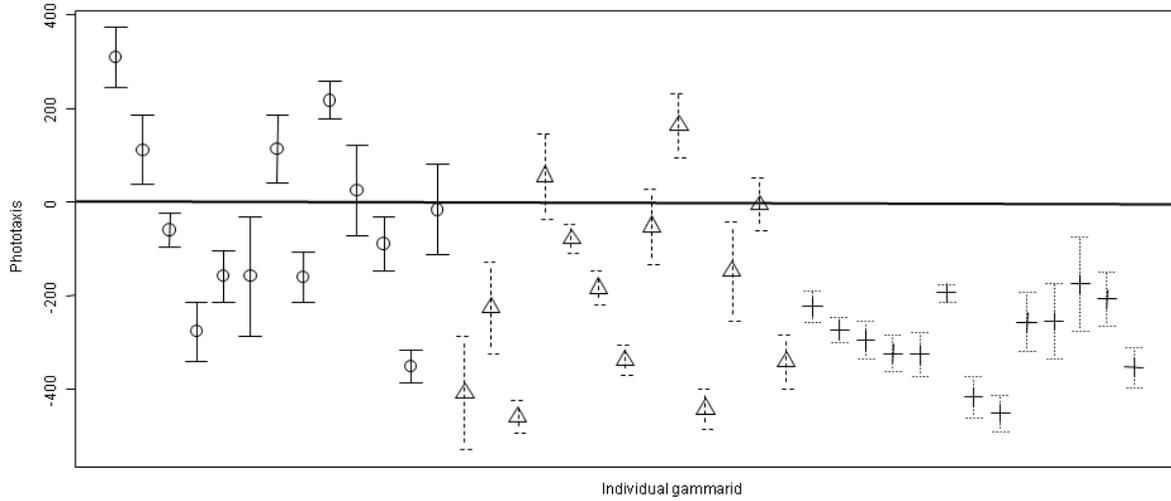
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507 Figure 2

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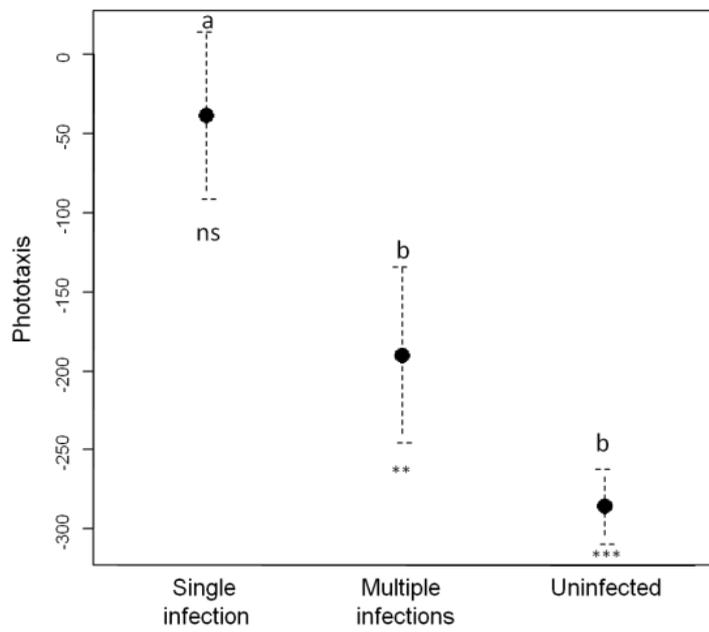
509 a)

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512 b)



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516 Fig. 2

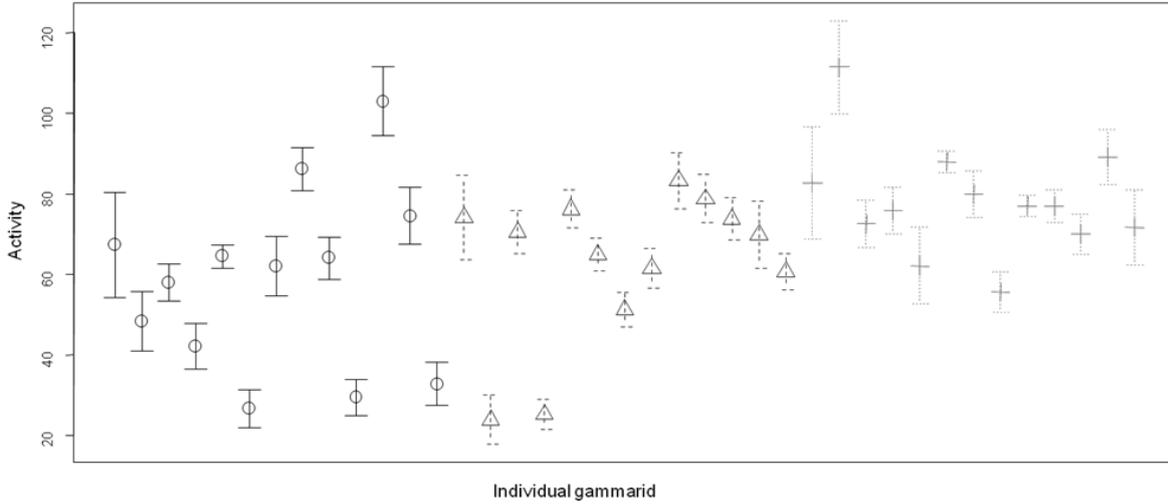
517 a): Phototaxis scores (time on light side minus time on dark side; values > 0 photophilic,
518 values < 0 photophobic) for individual gammarids: single-infected (circles), multiple- infected
519 (≥ 2 parasites, triangles), uninfected (crosses). Shown are mean values and SE for each tested
520 individual.

521 b): Phototaxis mean values and SE for the infection groups. Different letters above means
522 indicate significant difference between groups ($p < 0.05$). Symbols below means indicate
523 significant deviation from 0 (ns, $p > 0.05$, **; $p < 0.01$, ***; $p < 0.001$).

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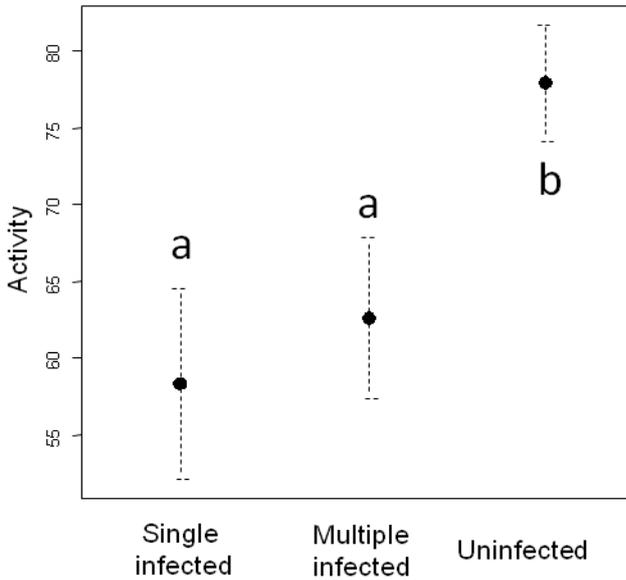
525 Figure 3

526 a)



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528 b)



529

530 Fig 3:

531 a) Activity, i.e. zone changes, for individual gammarids: Single-infected (circles), multiple-
532 infected (≥ 2 parasites, triangles), uninfected (crosses). Shown are mean values and SE for
533 each tested individual.

534 b) Activity mean values and SE for the infection groups. Different letters above means
535 indicate significant difference between groups ($p < 0.05$).

536 **Tables**

537

538 *Table 1: Results of linear mixed effect models (with individual as random factor). Effects of infection*

539 (uninfected, single-infected and multiple-infected) and experimental day (day) on gammarids'

540 phototaxis and activity.

541

dependent variable	interaction / fixed factor	N	Δ df	χ^2	P
Phototaxis	Infection group	39	2	11.732	0.002
	Infection group x days	39	2	0.712	0.700
	Days	39	1	3.058	0.080
Activity	Infection group	39	1	7.609	0.022
	Infection group x days	39	2	3.958	0.138
	Days	39	1	6.721	0.009

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545 *Table 2: Repeatability (R) with standard error (SE), 95% confidence intervals (CI)*
 546 *and p-values for phototaxis and activity for each infection group.*

547

Variable	Infection group	R	SE	CI	P
Phototaxis	Uninfected	0.192	0.098	0.006, 0.401	0.003
	Single-infected	0.382	0.118	0.127, 0.593	<0.001
	Multiple-infected	0.460	0.122	0.185, 0.647	<0.001
Activity	Uninfected	0.198	0.101	0.020, 0.406	0.002
	Single-infected	0.528	0.121	0.238, 0.710	<0.001
	Multiple-infected	0.496	0.126	0.195, 0.694	<0.001

548