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1 **High food quality increases *Gammarus pulex* (Crustacea: Amphipoda)**
2 **infection by the acanthocephalan parasite *Pomphorhynchus laevis***

3

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27 survival, growth, behavior

28 **Abstract**

29 Parasitism is one of the most important but largely neglected processes in ecosystems.
30 However, parasites are involved in a majority of trophic links in food webs, playing in turn a
31 major role in community structure and ecosystem processes. Several studies have shown that
32 higher nutrient availabilities in ecosystems tended to increase the prevalence of parasites. Yet,
33 most of these studies focused on resources availability, and those investigating resources
34 quality are scarce. In this study, we tested the impact of the quality of host food resources on
35 infection by parasites, as well as on the consequences for the host. Three resources were used
36 for feeding individually *Gammarus pulex* (Crustacea: Amphipoda) experimentally infected or
37 not with the acanthocephalan species *Pomphorhynchus laevis*: microbially-conditioned leaf
38 litter without phosphorus input (standard resource), microbially-conditioned leaf litter
39 enriched in phosphorus, and microbially-conditioned leaf litter without phosphorus input but
40 complemented with additional inputs of benthic diatoms rich in both phosphorus and
41 eicosapentaenoic acid (EPA). During the experiment, infection rate, parasite load, hosts'
42 survival, and parasite-mediated behavioral traits presumably implied in trophic transmission
43 were measured (refuge use, geotaxis and locomotor activity). The resources of higher quality,
44 regardless of the infection status, reduced gammarids mortality and increased gammarids
45 growth. In addition, higher quality resources increased the proportion of infected gammarids,
46 and led to higher cases of multi-infections. While slightly modifying the geotaxis behavior of
47 uninfected gammarids, resources quality did not modulate parasite impacts on hosts'
48 behavior. Finally, for most parameters, consumption of algal resources had a greater impact
49 than phosphorus-enriched leaf litter. Manipulation of resources quality therefore deeply
50 affected host-parasite relationships, a result stressing the need for future research to
51 investigate *in natura* the relationships between resources availability, resources quality, and
52 parasites prevalence.

53

54 **1. Introduction**

55 Parasitism is certainly one of the most important but largely neglected processes in
56 ecosystems (Lafferty *et al.*, 2008). Parasites, ranging from bacteria and protozoans to
57 metazoan species such as crustaceans or nematodes, use their hosts as providers of resources
58 and habitats. Recent studies suggested that parasites may represent at least half of species on
59 Earth, and could be involved in 75% of the trophic links in food webs (Dobson *et al.*, 2008).
60 To date, numerous studies have investigated the often complex life cycles of parasites and the
61 physiological mechanisms involved in hosts manipulation. Far less is known on the impacts
62 of environmental stressors on parasites success and, in turn, on the impacts of parasites on
63 food webs and ecosystem processes (Moore *et al.*, 2002; Labaude *et al.*, 2015a).

64 In all ecosystems, parasites' hosts are simultaneously exposed to several environmental
65 stressors, either from natural or anthropic origins, and data on the interactive impacts of these
66 stressors and parasites are clearly lacking. Among the different stressors that hosts might be
67 exposed to, resources availability is certainly one of the most common (Power, 1992). Several
68 studies have investigated the impact of resources quantity on host-parasite interactions
69 (Wright & Gompper, 2005; Civitello *et al.*, 2015). Some authors also explained current
70 increases in parasitic and infectious diseases by increased nutrient loading in the environment
71 (Johnson *et al.* 2010). Yet, in numerous ecosystems, resource quality has been shown as far
72 much important than resource quantity for understanding organisms' physiology, food web
73 structure, and ecosystem functioning (Elser *et al.*, 1998; Frost & Elser, 2002; Marcarelli *et al.*
74 2011). Although investigating resources quality parameters instead of quantifying resources
75 availability might be essential for understanding host-parasite interactions, data on this topic
76 remain extremely scarce.

77 Several parameters are generally considered for describing resources quality. Resources
78 elemental content, generally expressed as resources carbon (C): nitrogen (N): phosphorus (P)
79 ratios, have regularly been shown to control consumers' life history traits, especially for

80 herbivorous (Elser *et al.*, 2001, Schade *et al.*, 2003) or detritivorous (Danger *et al.*, 2013,
81 González *et al.*, 2014, Fuller *et al.*, 2015) species. This approach, called Ecological
82 Stoichiometry (Sterner & Elser, 2002), specifically investigates the consequences of
83 elemental imbalances between consumers' requirements and elements available in resources.
84 Since parasites rely on their hosts for resource acquisition, optimal elemental composition of
85 resources for an infected host might fulfill both parasites requirements but also host minimal
86 requirements for staying alive (Aalto *et al.*, 2015 ; Bernot & Poulain, 2018). In a recent
87 opinion paper, Bernot & Poulain (2018) highlighted the interest of ecological stoichiometry as
88 a pertinent framework for studying feedbacks of parasites on the environment as well as the
89 effect of the environment on parasites and diseases. Indeed, elemental requirements of
90 infected hosts might thus largely differ from those of non-parasitized ones (Frost *et al.*,
91 2008a), and, in turn, greatly alter the response of infected organisms to resources elemental
92 quality (Frost *et al.*, 2008b; Aalto & Pulkkinen, 2013). For example, Frost *et al.* (2008b)
93 showed that *Daphnia* fed with phosphorus rich resources had higher infection rates and
94 parasite loads than those fed with phosphorus-poor resources, this observation being
95 explained by a phosphorus-limitation of parasites' growth rates. Beyond a quantity aspect, an
96 indicator of resources quality is their lipid profiles, especially resource contents in
97 polyunsaturated fatty acids (Arts *et al.*, 2009). Indeed, some of these fatty acids are
98 considered as essential for consumers, since they cannot be synthesized or at least not in
99 sufficient amounts to fulfill the requirements of consumers (Arts *et al.*, 2009). Availability of
100 long chain PUFA in resources have been regularly reported as controlling consumers' growth
101 and/or reproduction, especially in aquatic ecosystems where they can mainly be found in
102 certain algal taxa (e.g. diatoms) or some protozoans (Müller-Navarra *et al.*, 2004; Bec *et al.*,
103 2006). Moreover some long chain PUFA such arachidonic acid and eicosapentaenoic acid
104 serve as biosynthetic precursor of eicosanoids known to be signalling molecules in many
105 physiological processes, mostly related to reproduction and immunity (Stanley-Samuelson

106 2006). This lead Schlotz et al (2013) to hypothesize that a high dietary supply with PUFA
107 may result in more pronounced immune response to the parasite resulting in increased
108 resistance to infection.

109 Among the numerous invertebrate species inhabiting freshwater ecosystems, the
110 crustacean *Gammarus pulex* (Crustacea: Amphipoda) represents an interesting biological
111 model for studying responses of host-parasite interactions to changes in resources quality.
112 This species is generally found in small headwater streams, and is particularly sensitive to
113 environmental changes (Maltby *et al.*, 2002). It also constitutes a host for many parasitic
114 species ranging from bacteria to macro-parasites such as helminths (Grabner, 2017). In
115 particular, several acanthocephalan species, such as *Pomphorhynchus laevis*, use gammarids
116 as intermediate hosts (Crompton & Nickol, 1985; Kennedy, 2006). These trophically-
117 transmitted parasites are well-known for inducing phenotypic modifications in their
118 intermediate hosts, making them more vulnerable to predation by definitive host species
119 (Bakker *et al.*, 1997). By this way, parasites increase the probability of completing their life
120 cycle. A few studies have shown that the presence of acanthocephalan parasites might alter
121 the response of gammarids to contaminant exposure, increasing their mortality (Brown &
122 Pascoe, 1989) and decreasing their antitoxic defenses (Marcogliese & Pietrock, 2011;
123 Gismondi *et al.*, 2012). More recent studies showed that increased temperature and parasite
124 infection additively reduced gammarids leaf litter consumption (Labaude *et al.*, 2017a).
125 Finally, the only study to our knowledge starting to investigate the effect of resources quality
126 was carried out by Labaude *et al.* (2015b), testing the influence of diet protein content on the
127 parasite load and on host behavioral modifications. Their results mainly showed that healthier,
128 well fed-gammarids exhibited higher infection intensity without any significant alteration of
129 hosts' behaviors.

130 Following the river continuum concept (RCC; Vannote *et al.*, 1980), headwater streams,
131 where can be found the detritivorous invertebrate *G. pulex*, are generally described as small

132 streams flowing in forested areas (or at least with a large canopy cover), at least in temperate
133 watersheds from the northern hemisphere. Their functioning is generally related to
134 allochthonous inputs of terrestrial detritus (leaf litter, dead wood, soil leachates...), these
135 detritus representing the main energy and nutrient source at the basis of food webs. Yet, due
136 to their small size and the anthropised surfaces they drain, these streams have long been
137 physically and chemically altered, especially when they are situated in agricultural contexts
138 (Moore & Palmer, 2005). In particular, headwater streams that are generally naturally quite
139 oligotrophic can receive large inputs of nutrients, such as nitrogen and/or phosphorus
140 (Carpenter *et al.*, 1998; Sims *et al.*, 1998; Peterson *et al.*, 2001). Moreover, a large proportion
141 of these streams have been or are currently submitted to morphological changes as well as a
142 degradation of the riparian corridors (Naiman *et al.*, 2010), inducing in turn an increase in
143 instream algal production and a reduction of the relative contribution of allochthonous detritus
144 inputs in comparison with autochthonous primary production (Feio *et al.*, 2010). Thus,
145 headwater stream perturbations should lead to higher elemental quality of resources for
146 detritivorous invertebrates, microbial decomposers being able to immobilize dissolved
147 nutrients in their biomass (Cross *et al.*, 2003), but also permit to these invertebrates to access
148 higher amounts of algae, these ones furnishing essential compounds for detritivores' growth
149 and survival (Crenier *et al.*, 2017; Rollin *et al.*, 2018).

150 In this study, we tested the effects of resources quality on the infection rate, parasite load,
151 hosts' survival, and parasite-mediated behavioral traits presumably implied in trophic
152 transmission (refuge use, geotaxis and locomotor activity of the surviving amphipods). Three
153 resources were used for feeding individually *G. pulex* experimentally infected or not by the
154 acanthocephalan species *P. laevis*: microbially-conditioned leaf litter without phosphorus
155 input (standard resource, S), microbially-conditioned leaf litter enriched in phosphorus (P),
156 and microbially-conditioned leaf litter without phosphorus input but complemented with
157 additional inputs of benthic diatoms rich in both phosphorus and eicosapentaenoic acid (EPA,

158 D). As reported in Rollin *et al.* (2018), we assumed that addition of algal resources to low
159 quality leaf litter will have a greater impact than phosphorus-enriched leaf litter, algae
160 bringing both phosphorus and essential fatty acids. We hypothesized that higher quality
161 resources, regardless of the infection status, will reduce *G. pulex* mortality (hypothesis 1) and
162 increase their growth (hypothesis 2). Higher quality resources, by increasing the energetic
163 status of hosts, is expected to increase parasite load (hypothesis 3), modify behavior of hosts
164 (hypothesis 4) and increase parasite impacts on hosts' behavioral changes (hypothesis 5).

165

166 **2. Materials and Methods**

167 **2.1. Sampling and maintenance**

168 Sycamore maple (*Acer pseudoplatanus*, Linnaeus) leaf litter was collected at abscission
169 from the riparian zone of a second-order forested stream located upstream of any direct
170 anthropogenic pollution (La Maix stream, Vosges Mountains, north-eastern France;
171 7°03'14.2"E; 48°29'24 9"N). This litter is commonly used in our experiments (see Danger *et*
172 *al.*, 2013; Arce-Funck *et al.*, 2016; Rollin *et al.*, 2017) because it is uncontaminated and its
173 physico-chemical characteristics are well described. Sycamore maple litter was dried in the
174 laboratory and stored at room temperature until use.

175 Water was collected in La Mance stream (6°02'24.4"E; 49°05'11 7"N), another headwater
176 forested stream presenting similar water physico-chemical characteristics compared to *G.*
177 *pulex* native stream ones (see below): pH = 8.2, conductivity = 580 $\mu\text{S cm}^{-1}$, acid neutralizing
178 capacity (ANC) = 4720 $\mu\text{eq L}^{-1}$, Cl^{-} = 13.3 mg L^{-1} , NO_3^{-} = 24.4 mg L^{-1} , SO_4^{2-} = 31.7 mg L^{-1} ,
179 PO_4^{3-} < 0.1 mg L^{-1} , Na^{+} = 6.5 mg L^{-1} , Ca^{2+} = 100 mg L^{-1} , K^{+} = 0.82 mg L^{-1} , and Mg^{2+} = 5.76
180 mg L^{-1} .

181 Specimens of *G. pulex* were sampled in April 2015 by kick-sampling technique in a small
182 tributary of the Suzon river (Burgundy, eastern France; 47°24'12.6"N ; 4°52'058.2"E), an
183 unpolluted forested stream. Over the past 20 years, the acanthocephalan parasite *P. laevis* was

184 never found in this stream, the *G. pulex* population in place is thus naïve for this parasite (see
185 Labaude *et al.*, 2015b). During the sampling, only males *G. pulex* were kept because the
186 experimental parasite infection was proved to be more efficient in males than in females
187 (Franceschi *et al.*, 2008). No noticeable infected gammarids were found. The animals were
188 placed in plastic coolers and rapidly transported to the laboratory, where they were sorted to
189 keep only one homogenous size. Individuals were kept in groups of 500 organisms in aerated
190 aquaria (37 x 55 x 10 cm) filled with La Mance water and acclimatized to laboratory
191 conditions (15°C ± 1; 12:12 h light:dark cycle) for 10 days prior to experimental infections.
192 Sycamore maple leaves were provided *ad libitum* as food resource.

193 Parasite eggs were collected from five naturally-parasitized chubs (*Squalius cephalus*)
194 caught in the Vouges river (Burgundy, eastern France, 47°9'34.36" N; 5°9'2.50" E). Fish
195 were anaesthetized, killed and dissected within 24 h after collection. Female parasites were
196 immediately taken from the intestines of the fish. Parasite eggs were obtained after dissection
197 of ten *P. laevis* gravid females. The taxonomy of parasites was verified by genetic analyses as
198 described in Franceschi *et al.* (2008). Sampled eggs were pooled in La Mance water to obtain
199 a concentrated but homogenous solution (1 mL). The number of eggs by microliter was
200 counted using a microscope on ten replicates.

201

202 **2.2. Experimental design**

203 Six combinations were studied in a fully balanced 3 × 3 factorial design (Fig. 1). Three
204 resource qualities (see 2.3.) were tested cross wise with the three parasite conditions related to
205 laboratory infestation procedure and then egg exposure/infection status.

206 The three tested resources qualities were i) conditioned maple leaves without P addition
207 (Standard litter, S, low quality treatment) or ii) with P addition (P, medium quality treatment)
208 or iii) with diatom supplementation using agarose pellets (D; in this case P and PUFA was
209 supplied by diatoms; high quality treatment; see Rollin *et al.*, 2018).

210 The three parasite conditions were: a) unexposed gammarids (Ue), b) exposed but uninfected
211 gammarids (Ui), and c) exposed and successfully infected gammarids (I). For statistical
212 analysis Ui and I conditions were also regrouped as exposed condition (E) and then compared
213 to Ue.

214

215 **2.3. Resource treatments**

216 Food resources were prepared in large quantities following the procedure described in
217 Rollin *et al.* (2018).

218

219 *2.3.1. Leaf phosphorus manipulation*

220 To have marked differences in stoichiometric leaves qualities, we selected maple leaves
221 as they contain initially low phosphorus content and exhibit a quite low carbon quality
222 (Hladyz *et al.*, 2009). Dried maple leaves were soaked for a few minutes in deionized water,
223 and 21,000 discs (20 mm in diameter) were then cut using a disc borer while avoiding the
224 main veins. These discs were placed by 200 in fine mesh size bags (mesh: 200 μm ; 15 x 25
225 cm) to avoid macroinvertebrate access. Bags were transferred in La Maix stream during 7
226 days for conditioning by microorganisms such as hyphomycetes and bacteria.

227 In the laboratory, discs were then rinsed using La Maix water and randomly introduced
228 into 105 Erlenmeyer flasks (500 mL, 200 discs per flask). Then, we added 200 mL of sterile
229 medium deprived from phosphorus in each flask. This treatment served as the standard food
230 treatment (low resource quality, S). In 35 randomly selected flasks, we introduced 4.64 mL of
231 KH_2PO_4 at 8.71 g phosphorus.L⁻¹, which served as the phosphorus implemented food
232 treatment (high quality resource 1, P; protocol fully described in Danger *et al.*, 2013). Discs
233 were incubated in the flasks for 4 days in the dark at 14°C on an orbital shaker, then sorted,
234 rinsed with deionized water, and stored at -20°C until use.

235

236

237 2.3.2 *Supplementation of diatoms in agarose pellets*

238 An axenic strain of the diatom *Nitzschia palea* (reference CPCC 160; high amount of
239 EPA) was grown during 2.5 weeks in four 5 L-Erlenmeyer flasks filled with 4 L of Combo
240 medium (Kilham *et al.*, 1998). This culture was maintained at 20°C in a temperature-
241 controlled chamber (50 µE, light/dark 16:8) under continuous shaking (150 rpm). Cells were
242 harvested by centrifugation (4,000 g, 10 minutes) at the end of the exponential growth phase
243 (2.5 weeks) and were freeze-dried and stored at –20°C until use.

244 Food source pellets were prepared using low gelling temperature agarose (Sigma
245 A9414) as a nutrient-free matrix, as fully described in Crenier *et al.* (2017). Briefly, agarose
246 was dissolved in glass bottles containing demineralized water (2%), heated in a microwave,
247 and placed in an agitated thermostatic water bath at 38°C. Risk of deterioration of fatty acids
248 due to excessive temperature was limited due to the low gelling point of agarose and the low
249 temperature applied. A mixture of 25 mg of diatoms and 1.6 ml of agarose solution was
250 introduced in a 2 ml Eppendorf® tube (to obtain a 50% carbon supply), homogenized using a
251 vortex and placed in the thermostatic bath until use to avoid agarose gelling. The pellets were
252 formed by injecting the homogenate in the holes of a Plexiglas® mold composed of 40 holes
253 (5 mm diameter x 2 mm high: 39.27 mm³). The pellets were unmolded after a few minutes
254 and were kept at –20°C in Petri dishes until use. Pellets were provided along with
255 unmanipulated leaves (S) to constitute a second high quality treatment (D), which was the
256 higher resource quality treatment. These pellets supplied EPA, phosphorus, and other
257 elements potentially of interest for organisms' development.

258

259 2.3.3. *Resource content analysis*

260 Four batches of 10 frozen leaf discs per treatment (S, P, see 2.3.1.), and four agarose
261 pellets were freeze-dried, ground, and weighed to the nearest 0.001 mg on a microbalance

262 (Perkin Elmer AD6 Autobalance, Perkin Elmer Corp., USA). A CHN elemental analyzer
263 (Carlo Erba NA2100, Thermo Quest CE International, Milan, Italy) was used to quantify
264 carbon and nitrogen contents in resources. Leaves and pellets were burned at 550°C for 12 h,
265 solubilized in 10 µl of suprapure HNO₃, and 5 mL of nanopure water were added. Phosphorus
266 content was quantified by spectrophotometry.

267 Pooled resource material (leaves, diatoms; n = 5) were used to perform fatty acid
268 analyses. According to the method proposed by Folch *et al.* (1957), a chloroform/methanol
269 solution was two times used to extract lipids. Then, fatty acids were converted into fatty acids
270 methyl-esters (FAMES) by acid catalyzed transesterification and analyzed on an Agilent
271 TechnologiesTM 6 850 gas chromatograph. FAMES were identified by comparing retention
272 times with those obtained from Supelco[®] and laboratory standards, and quantified against
273 internal standards (13:0).

274

275 **2.4. Infection procedure of *G. pulex***

276 Experimental infections were performed following the procedure described in Franceschi
277 *et al.* (2008) and Labaude *et al.* (2015b). First, gammarids were starved during 24h in
278 individual 60 ml glass dishes (15°C ± 1; 12:12 h light:dark cycle) to avoid cannibalism and
279 maximize gammarids feeding during infection procedure. Second, gammarids were pooled by
280 two in glass dishes (6 cm diameter) containing 50 mL of La Mance water at 15 ± 1°C. Then, a
281 conditioned maple leaf disk (S, see 2.3.1.), on which 100 *P. laevis* eggs were previously
282 deposited, was provided in each dish. Gammarids were allowed to feed on these disks for 48
283 h, thus initiating the infection procedure, i.e. *G. pulex* exposure (Exposed, E). In the same
284 experimental conditions, a control group, consisting of unexposed organisms (S leaf disc but
285 without parasite eggs, Ue), was performed to control for potential confounding effects of
286 infection procedure on gammarid endpoints. Overall, 1014 gammarids were exposed to
287 parasite eggs and 330 were unexposed in the same conditions.

288 After this infection step, living gammarids were rinsed and were placed individually in
289 new dishes in the same experimental conditions. Then, the three resource quality treatments
290 were randomly allocated for exposed and unexposed gammarids (338 and 110 gammarids per
291 food quality treatment, respectively; see table 1). Dead organisms (n=62) and gammarids
292 naturally infected by other parasite species (n=11) during the infection procedure were
293 removed from the analyses (differences between the two first column in Table 1).

294

295 **2.5. Experimental setup**

296 After the infection period, male gammarids were photographed for initial size
297 measurement at the beginning of the experiment (see 2.6.1.; ANOVA; no significant size
298 differences between treatments were observed; Mean size: 10.39 ± 0.11 mm; $p > 0.05$). The
299 experiment was conducted for 110 days in a natural unpolluted water at $15 \pm 1^\circ\text{C}$ under a
300 12:12 h light:dark cycle, which represents conditions close to gammarids optimum for growth
301 and survival. Each replicate consisted of a 60-mL glass dish containing 50 mL of La Mance
302 water, a male *G. pulex* (unexposed or exposed to parasite eggs, and then successfully infected
303 or not), and the corresponding food resource (1 disc of maple leaf with or without phosphorus
304 addition and with or without agar pellet). To limit chemical variations and to ensure that *G.*
305 *pulex* were fed *ad libitum*, water and food resources were renewed weekly.

306 Six weeks after the first infected *G. pulex* was observed (day 42), all dead gammarids were
307 dissected under a stereomicroscope to verify their infection status. Gammarids in which
308 parasites failed to develop are referred as uninfected (Ui) in the following parts. After 110
309 days, gammarids were photographed prior to the behavioral assays. This 110-day period
310 appeared to be a good compromise between measuring survival for a long enough time and
311 getting enough animals to perform behavioral tests. After behavioral assays, gammarids were
312 sacrificed and dissected under a stereomicroscope to determine their infection status (infected
313 or uninfected) and their parasite load.

314

315 **2.6. Endpoints**

316 *2.6.1. Gammarids survival*

317 The number of dead gammarids was recorded daily. The date of death of each individual
318 was noted and dead gammarids were immediately photographed for size assessment (see
319 2.6.2.) and dissected to assess the presence and the number of parasites (see 2.6.4.).

320

321 *2.6.2. Gammarids size and growth rate*

322 Gammarids were photographed under a stereomicroscope at the day they were found dead
323 or at the day 110 (end of the experiment). The height of their fourth coxal plate was measured
324 using SigmaScan Image Analysis Version 5.000 (SPSS Inc, Chicago, IL, USA). This metric is
325 a reliable proxy for gammarids size (Bollache *et al.*, 2000) and is widely used. To determine
326 gammarids body length (mm), we used the allometric relation established by Labaude (2016)
327 for males *G. pulex* coming from the same sampling site [body length (mm) = $4.33 \times$ fourth
328 coxal plate length (mm); $R^2 = 0.61$, $p < 0.0001$].

329 Individuals growth rates ($\text{mm} \cdot \text{mm} \cdot \text{day}^{-1}$) were calculated by dividing the difference between
330 the final size and the initial size by the initial size multiplied by the number of days between
331 the two measures.

332

333 *2.6.3. Behavioral assays*

334 The day after the end of the experiment, we assessed shelter use of each living
335 gammarids. The second day, we measured geotaxis of the same gammarids, followed the third
336 day by locomotor activity. For all of behavioral assays, blind recordings were ensured by
337 labelling testing device with a number giving no idea about the treatment to which gammarids
338 were belonging.

339 The sheltering behavior of individual gammarids was recorded by quantifying refuge use
340 according to Dianne *et al.* (2014). Sheltering behavior assays were performed on 75
341 unexposed *G. pulex* (Ue; 15, 31 and 29 for S, P, and D resource treatments, respectively) and
342 20 infected *G. pulex* (I; 1, 6, and 13 for S, P and D, respectively). Gammarids were
343 individually placed in boxes (10.5 × 16 cm) filled with 250 mL of La Mance water. At one
344 extremity, each box was containing an opaque refuge, consisting of half a saucer terracotta
345 pot (8.5 cm of diameter) with a 1 cm² hole in the convex part. After 5 minutes of
346 acclimatization, the gammarid position was recorded every 3 min during 1 h and scored as 1
347 when outside or 0 when inside the refuge. The cumulated score of shelter use therefore ranged
348 from 0 (always inside the refuge) to 20 (always outside) for each individual.

349 Geotaxis was estimated as the average vertical position of individuals in the water
350 column according to Labaude *et al.* (2017b). In total, geotaxis assays were performed on 75
351 unexposed *G. pulex* (Ue; 15, 31 and 29 for S, P and D resource treatment, respectively) and
352 20 infected *G. pulex* (I; 1, 6 and 13 for S, P and D, respectively). After 110 days of exposure,
353 gammarids were individually placed in a 500 ml-graduated cylinders (high: 35 cm; diameter:
354 6 cm) vertically divided in five zones of equal height, filled with 250 mL of La Mance water.
355 In order to allow gammarids to choose their position without having to swim, the inner walls
356 of the cylinders were covered with nylon mesh, making it possible to cling. This aspect was
357 important since the parasite is able to alter both the swimming and the clinging behaviors of
358 gammarids (Bauer *et al.*, 2005). Each cylinder was placed at 15°C in a temperature-controlled
359 chamber in which the light came only horizontally to avoid any confounding phototactic
360 reaction. After 3 minutes of acclimatization, the position of gammarids was recorded every 15
361 s for 5 min and scored from 1 (bottom) to 5 (top compartment). The cumulated geotaxis score
362 therefore ranged from 20 (always in the bottom zone) to 100 (always in the top zone) for each
363 individual.

364 The locomotor activity was assessed following the method used by Sornom *et al.* (2010).
365 This method usually measures the proportion of time spent moving. In total, locomotor
366 activity assays were performed on 57 unexposed *G. pulex* (Ue; 14, 22 and 21 for S, P and D
367 resource treatment, respectively) and 13 infected *G. pulex* (I; 1, 6 and 6 for S, P and D,
368 respectively). We placed the gammarids individually in 8 cm diameter Petri dishes open to the
369 air and filled with La Mance water. Petri dishes were then placed in a hermetic box linked to a
370 digital video camera. After 5 minutes of acclimatization, individuals' behavior was video-
371 recorded under red light at 15 frames per second for 5 min. Locomotor activity was scored as
372 the proportion of time swimming. Below 15 mm.s^{-1} , moving gammarids were considered
373 crawling rather than swimming.

374

375 2.6.4. Prevalence and parasite load

376 All gammarids were dissected under stereomicroscope ($\times 6$ to $\times 40$ magnification)
377 either the day individuals were found dead or at the end of the experiment. This step
378 permitted to remove gammarids infected by other parasites than *P. laevis*, define the status of
379 exposed *G. pulex* (infected, uninfected), the parasite load (number of parasites in one *G.*
380 *pulex*) and the prevalence of parasite (% of infected *G. pulex*) in each experimental condition.

381

382 2.7. Statistical analyses

383 The first infected gammarid was observed at day 47. Thus, two datasets were used for
384 survival rate and parasite load statistical analysis to integrate or not the delay of infection in
385 the analysis (from day 0 to 110 vs from day 47 to 110). Indeed, before 47 days of experiment,
386 it was not always possible to identify if gammarids were infested or not, due to the too low
387 development of parasites. For other endpoints, only the results obtained at the end of the
388 experiment were considered.

389 Statistical analyses were conducted using R software (R Core Team, 2016) to the 5%
390 level of significance. Survival data were analyzed by Kaplan-Meier survival curves and log-
391 rank tests were performed to compare curves and determine the effects of infection status and
392 resource quality on *G. pulex* survival rate throughout the experiment: from day 0 to 110
393 [difference between exposed and unexposed] and from day 47 to 110 [difference between
394 infected and uninfected. In the last case, mortality before days 47 (first infection detected)
395 was not considered]. As the number of infected gammarids was very low, log-rank tests were
396 also performed (day 47 to 110) by pooling infected and uninfected gammarids data or data of
397 gammarids fed on S, P and D resources to better assess the global effect of food resource or
398 effect of infection status, respectively. Bonferroni adjustments were performed on log-rank
399 tests to determine effects of resource quality and infection status on survival.

400 Due to the extremely low survival in infected-S treatment (n=1), only main effects were
401 testable for gammarids growth size and behavioral responses. Influence of food quality and
402 infection status on *G. pulex* growth was tested using Kruskal-Wallis tests, and post hoc tests
403 were performed on main effects (Mann-Whitney U-test and Bonferroni adjustment).

404 A test for proportion comparison permitting the calculation of 95% confidence intervals
405 was used to assess the effect of resource quality on the proportion of infected gammarids.
406 Significance between differences were assessed using confidence intervals. A multiple
407 Poisson regression model was used to investigate the effect of resource quality on individual
408 parasite load. In the analyses, the S resource treatment was considered as reference levels.

409 Kruskal-Wallis tests were used to assess the effect of resource quality on each behavior
410 score (geotaxis, shelter use, locomotor activity). Cliff's deltas (Cliff, 1996) were calculated to
411 evaluate the behavior score differences of infected (I) and unexposed (Ue) organisms fed with
412 the different resource qualities (S, P, D). Then, effect sizes for each score between unexposed
413 (Ue) and infected (I) organisms were also compared for P and D resource qualities (not for S
414 due to the too low number of surviving organisms). Cliff's delta, robust to non-normally

415 distributed data, is a scale-less parameter ranging from -1 to 1. It is used to represent the size
416 of the effect and the direction of this effect size difference. Significance between differences
417 was assessed using confidence intervals. The R-package 'orddom' (version 3.1) was used to
418 calculate Cliff's deltas medians and 95% confidence intervals.

419

420

421

422 **3. Results**

423 **3.1. Resource compositions**

424 Low resource quality leaves (S) contained 0.36 ± 0.08 mg phosphorus.g⁻¹ and
425 manipulated leaves (P) contained 1.24 ± 0.16 mg phosphorus.g⁻¹. The dominant fatty acids in
426 conditioned leaves (S or P) were palmitic acid (16:0), oleic acid (18:1 ω 9), linoleic acid
427 (18:2 ω 6), and α -linolenic acid (18:3 ω 3), whereas long chain PUFAs (number of Cs \geq 20)
428 were totally lacking from these resources.

429 Agarose pellets contained 1.95 ± 0.58 mg phosphorus.g⁻¹. In diatoms the major fatty
430 acids were palmitic acid (16:0), palmitoleic acid (16:1 ω 7), and EPA (20:5 ω 3). As mentioned
431 in Rollin *et al.* (2018), these compounds accounted for more than 65% of total fatty acids
432 (16:0 = 28.2%, 16:1 ω 7 = 21%, and 20:5 ω 3 = 16.9%). Docosahexaenoic acid (22:6 ω 3)
433 represented 2.2% of diatoms fatty acids.

434

435 **3.2. *G. pulex* survival**

436 After 47 days of exposure (day of the first observed parasite), the survival of gammarids
437 was high, ranging from 81.7 % in exposed (E; Ui+I)-P treatment to 96 % in Ue-D treatment
438 (Table 1). At 110 days, survival rates were low, ranging between 15.4 % in Ue-S treatment
439 and 33.9 % in E-D treatment. The highest *G. pulex* survival was observed when organisms fed
440 on the highest resource qualities (D>P>S), regardless of the infection status (Table 1).

441 The Log-rank global comparison performed on *G. pulex* exposed or not to *P. laevis* eggs (Fig
442 2a., day 0 to 110) and fed on one of the three resource qualities reports highly significant
443 differences in gammarid survival rates ($\text{Chi}^2 = 30.9$, d.f. = 5, $p = 9.68 \times 10^{-6}$). Two by two log-
444 rank tests with Bonferroni adjustment showed that survival in E-D and Ue-D treatments
445 (highest quality resource) was significantly higher than survival in E-S and Ue-S treatments
446 (the lower quality resource) [E-D vs E-S: $\text{Chi}^2 = 21.6$, d.f. = 1, $p = 3.82 \times 10^{-6}$; E-D vs Ue-S:
447 $\text{Chi}^2 = 9$, d.f. = 1, $p = 0.0027$; Ue-D vs Ue-S: $\text{Chi}^2 = 11$, d.f. = 1, $p = 9 \times 10^{-4}$; Ue-D vs E-S:
448 $\text{Chi}^2 = 17.3$, d.f. = 1, $p = 3.21 \times 10^{-5}$]. P resource led to intermediate but not significantly
449 different survival.

450 Among exposed gammarids, between day 47 and day 110, the survival rate ranged from
451 10 % in I-S treatment to 41.8% in Ui-D treatment. Infected gammarids exhibited a
452 significantly lower survival than unexposed and uninfected organisms (S: 10 % vs 17 and
453 24.9%; S: 18.4 % vs 33 and 36.7%; S: 22.2 % vs 30.6 and 41.8%; Table 1).

454 The Log-rank global comparison performed on *G. pulex* infected or not by *P. laevis* (Fig 2b,
455 day 47 to 110) fed with one of the three resource qualities also reports highly significant
456 differences in gammarid survival rates ($\text{Chi}^2 = 32.08$, d.f. = 5, $p = 4.09 \times 10^{-6}$). Two by two
457 log-rank test with Bonferroni adjustment showed that survival in Ui-S treatment was
458 significantly lower than survival in Ui-P and Ui-D treatments (Ui-S vs Ui-P: $\text{Chi}^2 = 9.4$, d.f. =
459 1, $p = 0.0022$; Ui-S vs Ui-D: $\text{Chi}^2 = 20.1$, d.f. = 1, $p = 7.33 \times 10^{-6}$) and that Ui-D treatment
460 had significantly higher survival than I-P and I-D treatments (Ui-D vs I-P : $\text{Chi}^2 = 12.2$, d.f. =
461 1, $p = 4.8 \times 10^{-4}$; Ui-D vs I-D: $\text{Chi}^2 = 8$, d.f. = 1, $p = 4.64 \times 10^{-3}$). No other differences were
462 found.

463 The effect of food resource was clearly significant, but due to a low infected gammarids
464 number, the effect of infection was not observed using the 2 by 2 log-rank. When log-rank
465 was performed on pooled infection status data (infected and uninfected; day 47 to 110), a
466 significant global effect of food treatment was observed ($\text{Chi}^2 = 16.9$, d.f. = 2, $p = 2.19 \times 10^{-4}$)

467 with gammarid fed with S resource showing significant lower survival compared to those fed
468 with P ($\text{Chi}^2 = 6.9$, d.f. = 1, $p = 0.00865$) and D resources ($\text{Chi}^2 = 16.4$, d.f. = 1, $p = 5.25 \times 10^{-5}$).
469 In the same way, when log-rank tests were performed on pooled food resource data (S, P
470 and D; day 47 to 110), a significant global effect of infection status was reported ($\text{Chi}^2 = 10.9$,
471 d.f. = 2, $p = 0.00433$), with uninfected gammarids showing significantly higher survival
472 compared to those infected by *P. laevis* ($\text{Chi}^2 = 9.4$, d.f. = 1, $p = 2.155 \times 10^{-3}$). Thus, survival
473 was influenced by both resource quality and infection status, but no interactive effect was
474 observed. No global survival differences were reported between unexposed and uninfected
475 gammarids ($\text{Chi}^2 = 3.4$, d.f. = 1, $p = 0.0638$) showing the absence of infestation procedure
476 impact.

477

478 **3.3. *G. pulex* growth**

479 Resource quality had a significant effect on *G. pulex* growth (Kruskal-Wallis; $\text{Chi}^2 =$
480 140.69 , d.f. = 2, $p = 2.2 \times 10^{-16}$; Fig. 3). Two by two Mann Whitney U-test with Bonferroni
481 adjustment revealed that size growth of *G. pulex* fed with D resource was significantly higher
482 than that of gammarids fed with P and S resources (D vs P: $\text{Chi}^2 = 2343.5$, d.f. = 1, $p = 2.2 \times$
483 10^{-16} ; D vs S: $\text{Chi}^2 = 1398$, d.f. = 1, $p = 2.2 \times 10^{-16}$) and that the growth of *G. pulex* fed with P
484 resource was significantly higher than that of gammarids fed with S resource ($\text{Chi}^2 = 2680$,
485 d.f. = 1, $p = 4.4 \times 10^{-8}$). On average, compared to S treatment, gammarids growth was
486 increased by 17 and 56.4 % in individuals fed with P and D resource, respectively.

487 Infection status also had a significant effect on *G. pulex* growth (Kruskal-Wallis,
488 resource quality: $\text{Chi}^2 = 9.66$, d.f. = 2, $p = 0.008$). The growth of infected gammarids was
489 similar regardless of the resource quality. Two by two Mann Whitney U-test with Bonferroni
490 adjustment revealed that the growth of *G. pulex* infected by *P. laevis* was significantly lower
491 than that of uninfected gammarids (I vs Ui: $\text{Chi}^2 = 3703$, d.f. = 1, $p = 0.002$). On average,

492 compared to uninfected gammarids, size growth of infected gammarids was reduced by 20.3
493 %.

494

495 **3.4. Prevalence and parasite load**

496 *G. pulex* infection success by *P. laevis* was significantly affected by resource quality,
497 both when considering all gammarids (dead and alive) taken after 47 days ($\text{Chi}^2 = 47.29$, d.f.
498 = 2, $p = 5.38 \times 10^{-11}$, Fig. 4a) and those still alive at the final date (110 days; $\text{Chi}^2 = 6.21$, d.f.
499 = 2, $p = 0.045$, Fig. 4b), the number of infected *G. pulex* increasing while increasing resource
500 quality.

501 Among the gammarids exposed to *P. laevis* eggs, 2.7 % [1.2-5.2], 11.4 % [8.2-14.4]
502 and 20.1 % [15.8-25.0] of *G. pulex* (mean % [95% confidence interval]) were infected and
503 alive after 47 days of feeding on S, P, and D resources, respectively (n=10, 38, and 63; Table
504 1). Based on the 95% confidence intervals, it can be deduced that infection success
505 significantly differed between the three resource qualities. At 110 days, only 1.7 % [0.04-8.5],
506 7.2 % [2.6-15] and 20.1 % [7.1-21.4] of gammarids, representing 1, 7 and 14 individuals,
507 were alive and infected in S, P and D treatment, respectively (Table 1).

508 Among infected *G. pulex*, amphipods fed with D resource had a significantly higher *P.*
509 *laevis* load when considering all individuals, dead or not (day 47 to 110; S as reference level;
510 $p = 2.73 \times 10^{-13}$, Fig. 4c), or only the surviving ones (day 110; S as reference level; $p = 0.012$,
511 Fig. 4d). P resource also significantly increased *P. laevis* load in individual gammarid but
512 only when all individuals, dead or not, were considered (day 47 to 110; S as reference level; p
513 = 4.13×10^{-6} , Fig. 4c). Considering all individuals, the relative proportion of infected
514 gammarids with only one *P. laevis* was reduced by a factor of 1.4 between S and P resource
515 and by a factor of 1.9 between S and D resources (by 2 and by 2.6 at final time, but only one
516 organism was present in infected-S treatment).

517

518 3.5. Behavior

519 Resource quality had a marginally significant effect on *G. pulex* geotaxis score (Kruskal-
520 Wallis; $\text{Chi}^2 = 6.15$, d.f. = 2, $p = 0.046$; Fig. 5a), but not on shelter use ($\text{Chi}^2 = 1.53$, d.f. = 2, p
521 = 0.47; Fig. 5b) or locomotor activity ($\text{Chi}^2 = 2.69$, d.f. = 2, $p = 0.26$; Fig. 5c). Two by two
522 Mann Whitney U-test with Bonferroni adjustment showed that mean geotaxis scores among
523 resource treatments were similar (mean values of 28.9, 36.8, and 38.6 for S, P, and D,
524 respectively), while Cliff's delta revealed a significantly higher geotaxis score in Ue-D and
525 Ue-P compared to Ue-S treatment (Fig. 6a.). Unexposed gammarids fed on higher quality
526 resource were significantly higher in the water column.

527 Compared to unexposed *G. pulex*, those infected with *P. laevis* showed a significantly
528 higher geotaxis score (Mann-Whitney; $U = 338$, d.f. = 1, $p = 1.652 \times 10^{-4}$; in mean 32.7 vs
529 53.6; Fig. 5a), a significantly higher shelter use score (Mann-Whitney; $U = 505.5$, d.f. = 1, $p =$
530 0.0244; in mean 6.7 vs 10.3; Fig. 5b), but no differences were reported for locomotor activity
531 (Mann-Whitney; $U = 340$, d.f. = 1, $p = 0.6504$; in mean 0.364 vs 0.378; Fig. 5b). Infected
532 gammarids thus tended to be higher in the water column and more often outside of the refuge.
533 However, when considering Cliff's delta approach, no significant differences were reported
534 between treatments.

535

536 4. Discussion

537

538 To date, only few studies evaluated the effect of stoichiometric quality of the resource,
539 excluding confounding factors (e.g. changes in microbial biomass, alteration of detrital carbon
540 quality), on the survival and life history traits of a detritivorous species (Danger *et al.*, 2013;
541 Arce-Funck *et al.*, 2016; Rollin *et al.*, 2018). Similarly, studies on the effect of stoichiometric
542 quality of the resource on parasitic infection are scarce and did not concerned detritivorous
543 hosts (Frost and Elser, 2008, Schlotz *et al.*, 2013), Stephens *et al.*, 2016).

544 In the present study, comparison of S (microbial conditioned maple leaves) and P
545 (phosphorus-manipulated conditioned maple leaves) resources treatment allowed
546 investigating stoichiometric effects due to the higher phosphorus content in food resource
547 (Danger *et al.*, 2013). In addition, a supplementation of leaf litter with diatom pellets (D) was
548 expected to greatly increase resource quality for consumers. Indeed, diatoms are easier to
549 assimilate than detritus, they show high elemental quality (higher phosphorus contents than
550 leaf litter), and contains high EPA concentration (Crenier *et al.*, 2017).

551 Resource quality was the main driver of *G. pulex* survival rates, infected or not by *P.*
552 *laevis*. Higher survival was related to higher quality of resources consumed by *G. pulex*
553 (S<P<D). Higher growth rates were also reported for *G. pulex* fed on higher resource quality.
554 These results are in accordance with several studies reporting a beneficial effect on aquatic
555 organisms' survival and growth rate of resources containing high phosphorus or EPA rates
556 (*Daphnia*: Makino *et al.*, 2002; Masclaux *et al.*, 2009; Schlotz *et al.*, 2012; Prater *et al.*, 2016;
557 *Oncorhynchus mykiss*: Lellis *et al.*, 2004; *Gammarus*: Kraufvelin *et al.*, 2006; Danger *et al.*,
558 2013; Gergs *et al.*, 2014; Crenier *et al.*, 2017; Rollin *et al.*, 2017). According to Rollin *et al.*
559 (2018), higher *G. pulex* growth rate when fed on higher resource quality was linked to a
560 higher molt frequency. This is in accordance with the growth rate hypothesis stating that
561 growth requires high phosphorus amounts, phosphorus being involved in the synthesis of
562 phosphorus-rich RNA necessary for ensuring organisms' growth (Elser *et al.*, 2003).
563 Beneficial effect of PUFA on gammarids' growth might be due to their inability to maintain
564 their PUFA content and/or to synthesize these essential compounds (Crenier *et al.*, 2017).

565 In the present study, *G. pulex* infected by *P. laevis* showed lower survival and lower
566 growth rates than those unexposed and uninfected. These results are in accordance with
567 previous studies (Lafferty *et al.*, 2008; Aalto & Pulkkinen, 2013; Labaude *et al.*, 2015b;
568 Labaude, 2016). Growth reduction could be related to the energetic costs of harboring a
569 parasite. Crustaceans infected by acanthocephalan parasites showed significant modifications

570 in their energetic reserves (Plaistow *et al.*, 2001; Gismondi *et al.*, 2012; Caddigan *et al.*,
571 2014), and isopods infected by acanthocephalans were reported to allocate about 21% of their
572 energy production to parasite growth, to the detriment of their own reproduction (Lettni &
573 Sukhdeo, 2010).

574 Leaving enough resources to their hosts might be a strategy selected in parasites,
575 allowing them to manipulate host behaviors (Maure *et al.*, 2013). According to this
576 hypothesis, when the host condition is lowered, a lower parasite exploitation is expected. In
577 the present study both low resource quality (S) and infection with *P. laevis* significantly
578 reduced *G. pulex* survival, but no clear interactive effects were identified. Survival was
579 systematically lowered for infected *G. pulex* compared to uninfected organisms whatever the
580 resource quality. In addition, the growth of infected gammarids was similar regardless of the
581 resource quality. Therefore, our results did not confirmed the hypothesis of Maure *et al.*
582 (2013), and were in accordance with Labaude *et al.* (2015b). Similarly, Frost *et al.* (2008)
583 showed that low phosphorus content in resource increased deleterious effects of bacterial
584 infection on *Daphnia* reproduction.

585 One of the important results of our study concerns the impacts food stoichiometric quality
586 had on *G. pulex* infection by the acanthocephalan species *P. laevis*: high resources qualities
587 were associated to higher prevalences. Several studies have shown that high nutrient
588 availabilities in ecosystems tended to increase the prevalence of parasites (Johnson *et al.*,
589 2010; Altman & Byers, 2014). However, most of these studies mainly considered resources
590 quantity, rarely resources quality. **Insertion ici de la dernière partie de ce paragraphe?** Our
591 results thus provide an argument for a positive effect of the quality of resources on a parasitic
592 infection. This in in line with results of Frost and Elser (2008), showing that the proportion of
593 *Daphnia* infected, as well as the number of parasites per *Daphnia*, were largely increased
594 when organisms fed on low Carbon/Phosphorus resources, or those of Stephens *et al.* (2016),
595 that showed on amphibian parasites that higher litter quality does not decrease susceptibility

596 to infection. Therefore, in these cases, higher amounts of energy in the host might permit a
597 better development of parasites, rather than allowing the host to increase its energy, allowing
598 better defenses (Arce-Funck *et al.*, 2016, Stephens *et al.*, 2016). Another interesting result is
599 the increase in the parasite load (number of *P. laevis* per gammarid) for organisms fed with
600 the highest quality resources (P and D). This result suggests that the higher energy contents of
601 gammarids fed with high quality resources, already observed in Arce Funck *et al.* (2016),
602 favored several parasites to co-exist in a single individual through reducing competition
603 between parasites. Interestingly, Schlotz et al (2013) also observed that infected daphnids by
604 the parasite *Pasteuria ramosa* showed the highest spore load per animal when fed a high
605 quality food. Even if the number of infected organisms at the end of the experiment preclude
606 strong statistical testing, the number of parasites per gammarid seemed unrelated to higher
607 mortality, the proportion of multi-infected gammarids remaining unchanged throughout the
608 experiment. This result is in accordance with the results of Franceschi *et al.* (2008) who did
609 not observe any effect of infection intensity on host survival. Finally, we showed that diatom
610 supplementation led to significantly higher infection rates than phosphorus-enriched resources
611 alone, suggesting that in ecosystems, consumption of such a high quality resource, bringing
612 both nutrients and PUFA, might strongly impact parasites development, at least more than
613 ingestion of leaf litter conditioned in streams with high nutrient loads. This part is particularly
614 important in a global context of degradation of riparian vegetation and canopy opening in the
615 vicinity of streams (Hladyz *et al.* 2011).

616 Despite the important effects of resources quality on host survival and growth, as well
617 as on parasite prevalence and infection intensity, our study did not reveal clear impacts of
618 resources quality on the modulation of parasites-induced behavioral changes of hosts. As
619 expected, after 110 days of experiment, *P. laevis* infection led to changes in hosts' geotaxis
620 and shelter use. Behavioral changes have long been described in this host-parasite interaction
621 (Bakker *et al.*, 1997; Bauer *et al.*, 2005; Lagrue *et al.*, 2007). These changes, according to the

622 manipulation hypothesis (Holmes & Bethel, 1972), predispose manipulated intermediate hosts
623 to be preyed upon by definitive hosts, *i.e.* a fish. Such behavioral changes are mainly related
624 to parasite-induced changes in hosts' neurotransmitters production (Tain *et al.*, 2006, Perrot-
625 Minnot *et al.*, 2014). Maure *et al.* (2013) proposed the "host energetic resource constraint
626 hypothesis" (HERC) stating that a largely overlooked part of infected hosts' energy might be
627 used during the behavioral manipulation of the host. Following this hypothesis, higher
628 resources quality should have permitted the parasite to amplify the behavioral manipulation of
629 its host. The absence of such effect suggests that HERC is not applying in gammarids infected
630 with *P. laevis*. However, this result must be taken cautiously due to the very low statistical
631 power generated by the very low survival of infected gammarids fed with standard food
632 resources. Results of Franceschi *et al.* (2010) suggested a trade-off between *P. laevis* growth
633 rate and behavioral manipulation. However, working on the same host and parasite species,
634 Labaude *et al.* (2015b) did not report any effect of resource quality on parasite growth.
635 Finally, while resources quality had no amplification effect on parasite-mediated host
636 manipulation, resource quality had a small but significant effect on the geotaxis of uninfected
637 gammarids. As proposed by Arce Funck *et al.* (2016), who observed a change in (another)
638 behavior following increased food quality, the observed effect on geotaxis probably relies on
639 a higher energetic content of well-fed organisms.

640 To sum-up, this experiment permitted to highlight that higher quality resources,
641 regardless of the infection status, reduced *G. pulex* mortality (hypothesis 1 in the
642 introduction), while only uninfected gammarids exhibited higher growth rates when fed with
643 high quality resources (partial validation of hypothesis 2). In addition, higher quality resource
644 increased parasites' infection rates (verifying hypothesis 3). While slightly modifying the
645 geotaxis behavior of uninfected organisms (partially verifying hypothesis 4), resources quality
646 did not modulate parasites impacts on hosts' behavior (rejecting hypothesis 5). Finally, for
647 most parameters investigated, consumption of algal resources had a greater impact than

648 phosphorus-enriched leaf litter. Our experiment thus confirms that in addition to changes in
649 resource quantity, changes in resources quality might play a major role in the currently
650 observed development of parasites. This result stresses the need for future research to
651 investigate *in natura* the relationships between resources availability, resources quality and
652 parasites prevalence. In particular, it might be important to verify if rivers with higher algal
653 production (e.g. rivers with open canopies; Feio *et al.*, 2010) exhibit higher parasite
654 prevalence. Moreover, since all algal groups do not have the same biochemical quality (e.g.
655 chlorophytes or cyanobacteria lack EPA, Bec *et al.*, 2010), and since diatoms are
656 progressively replaced by chlorophytes or cyanobacteria along nutrient load gradients
657 (Leflaive *et al.*, 2015), stream eutrophication might lead first to increased parasite prevalence
658 until a decline related to shifts in algal communities. Finally, it would be interesting to
659 evaluate if parasites could modify the host feeding behavior (food selection) toward food with
660 high Phosphorus/algal contents, to allow them a better development. In this context, using the
661 conceptual frameworks of trophic ecology, such as ecological stoichiometry (Bernet &
662 Poulain, 2018) might permit to understand in more depth the host-parasite interaction in
663 ecosystems, and the impact the anthropic-driven changes might have on these interactions.
664 Such research might be especially important when considering the importance of parasites in
665 ecological processes, and, *in fine*, on ecosystem services (Lafferty and Kuris, 2012).

666

667

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672

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674 **References**

- 675 Aalto SL & Pulkkinen K, 2013. Food stoichiometry affects the outcome of *Daphnia*–parasite
676 interaction. *Ecology and evolution* 3(5), 1266-1275.
- 677 Aalto SL, Decaesestecker E & Pulkkinen K, 2015. A three-way perspective of stoichiometric
678 changes on host-parasite interactions. *Trends in parasitology* 31, 333-340.
- 679 Altman I & Byers JE, 2014. Large-scale spatial variation in parasite communities influenced
680 by anthropogenic factors. *Ecology* 95, 1876–1887
- 681 Arce-Funck J, Crenier C, Danger M, Cossu-Leguille C, Guéroid F & Felten V, 2016.
682 Stoichiometric constraints modulate impacts of silver contamination on stream detritivores:
683 an experimental test with *Gammarus fossarum*. *Freshwater Biology* 61, 2075-2089.
- 684 Arts, MT, Brett, MT, & Kainz, M (Eds.), 2009. *Lipids in aquatic ecosystems*. Springer
685 Science & Business Media.
- 686 Bakker T, Mazzi D & Zala S, 1997. Parasite-induced changes in behavior and color make
687 *Gammarus pulex* more prone to fish predation. *Ecology* 78(4), 1098-1104.
- 688 Bauer, A, Haine, ER, Perrot-Minnot, MJ, & Rigaud, T, 2005. The acanthocephalan parasite
689 *Polymorphus minutus* alters the geotactic and clinging behaviours of two sympatric
690 amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeseli*. *Journal*
691 *of Zoology* 267(1), 39-43.
- 692 Bec A, Martin-Creuzburg D & von Elert E, 2006. Trophic upgrading of autotrophic
693 picoplankton by the heterotrophic nanoflagellate *Paraphysomonas sp.* *Limnology and*
694 *Oceanography* 51(4), 1699-1707.
- 695 Bec A, Perga M-E, Desvillettes C & Bourdier G, 2010. How well can the fatty acid content of
696 lake seston be predicted from its taxonomic composition? *Freshwater Biology* 55 (9):
697 1958–1972.
- 698 Bernot RJ & Poulain, R, 2018. Ecological stoichiometry for parasitologists. *Trends in*
699 *Parasitology* 34(11), 928-933.

700 Bollache L, Gambade G, Cézilly F, 2000. The influence of micro-habitat segregation on size
701 assortative pairing in *Gammarus pulex* (L.) (Crustacea, Amphipoda). Archiv für
702 Hydrobiologie 147, 547–558.

703 Brown AF & Pascoe D, 1989. Parasitism and host sensitivity to cadmium: An
704 acanthocephalan infection of the freshwater amphipod *Gammarus pulex*. Journal of
705 Applied Ecology 26, 473-487

706 Caddigan SC, Barkauskas RT & Sparkes TC, 2014. Intra-population variation in behavior
707 modification by the acanthocephalan *Acanthocephalus dirus*: are differences mediated by
708 host condition? Parasitology Research 113, 4307–4311.

709 Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN & Smith VH, 1998.
710 Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological*
711 *applications*, 8(3), 559-568.

712 Civitello DJ, Penczykowski RM, Smith AN, Shocket MS, Duffy MA, Hall SR, 2015.
713 Resources, key traits, and the size of fungal epidemics in *Daphnia* populations. Journal of
714 Animal Ecology 84(4), 1010-1017.

715 Cliff N, 1996. Ordinal methods for behavioral data analysis. Lawrence Erlbaum Associates,
716 Inc.

717 Crenier C, Arce-Funck J, Bec A, Perrière F, Billoir E, Leflaive J, Guérolde F, Felten V &
718 Danger M, 2017. Minor food sources can play a major role in secondary production in
719 detritus-based ecosystems. *Freshwater Biology* 62, 1155-1167.

720 Crompton DWT & Nickol BB. (Eds.), 1985. Biology of the Acanthocephala. Cambridge
721 University Press.

722 Cross WF, Benstead JP, Rosemond AD & Bruce Wallace BJ, 2003. Consumer-resource
723 stoichiometry in detritus-based streams. *Ecology Letters* 6(8), 721-732.

724 Danger M, Arce-Funck J, Devin S, Heberle J & Felten V, 2013. Phosphorus content in
725 detritus controls life-history traits of a detritivore. *Functional Ecology* 27, 807–815.

726 Dianne L, Perrot-Minnot M-J, Bauer A, Guvenatam A, Rigaud T, 2014. Parasite-induced
727 alteration of plastic response to predation threat: increased refuge use but lower food intake
728 in *Gammarus pulex* infected with the acanthocephalan *Pomphorhynchus laevis*.
729 International Journal of Parasitology 44, 211–6.

730 Dobson A, Lafferty KD, Kuris AM, Hechinger RF & Jetz W, 2008. Homage to Linnaeus:
731 How many parasites? How many hosts? Proceedings of the National Academy of Sciences
732 105 (1), 11482-11489.

733 Dutra BK, Fernandes FA, Lauffer AL & Oliveira GT, 2009. Carbofuran-induced alterations in
734 the energy metabolism and reproductive behaviors of *Hyaella castroi* (Crustacea,
735 Amphipoda). Comparative Biochemistry and Physiology Part C: Toxicology &
736 Pharmacology 149, 640–646.

737 Elser JJ, Hayakawa K & Urabe J, 2001. Nutrient limitation reduces food quality for
738 zooplankton: Daphnia response to seston phosphorus enrichment. Ecology 82, 898–903.

739 Elser JJ, Dobberfuhl D, MacKay NA & Schampel JH, 1996. Organism size, life history, and
740 N:P stoichiometry: towards a unified view of cellular and ecosystem processes. BioScience
741 46, 674–684.

742 Feio MJ, Alves T, Boavida M, Medeiros A & Graça MAS, 2010. Functional indicators of
743 stream health: a river-basin approach. Freshwater Biology 55(5), 1050-1065.

744 Franceschi N, Bauer A, Bollache L, Rigaud T, 2008. The effects of parasite age and intensity
745 on variability in acanthocephalan-induced behavioural manipulation. International Journal
746 for parasitology 38, 1161-1170.

747 Franceschi, N, Bollache, L, Cornet, S, Bauer, A, Motreuil, S, & Rigaud, T, 2010. Co-variation
748 between the intensity of behavioural manipulation and parasite development time in an
749 acanthocephalan–amphipod system. Journal of evolutionary biology 23(10), 2143-2150.

750 Frost PC & Elser JJ, 2002. Growth responses of littoral mayflies to the phosphorus content of
751 their food. Ecology Letters 5, 232–240.

752 Frost PC, Ebert D, & Smith VH, 2008a. Bacterial infection changes the elemental
753 composition of *Daphnia magna*. *Journal of animal ecology* 77(6), 1265-1272.

754 Frost PC, Ebert D & Smith VH, 2008b. Responses of a bacterial pathogen to phosphorus
755 limitation of its aquatic invertebrate host. *Ecology* 89(2), 313-318.

756 Fuller CL, Evans-White MA & Entekin SA, 2015. Growth and stoichiometry of a common
757 aquatic detritivore respond to changes in resource stoichiometry. *Oecologia* 177(3), 837-
758 848.

759 Gergs R, Steinberger N, Basen T & Martin-Creuzburg D, 2014. Dietary supply with essential
760 lipids affects growth and survival of the amphipod *Gammarus roeselii*. *Limnologica* 46,
761 109-115.

762 Gismondi E, Cossu-Leguille C, Beisel J-N, 2012. Does the acanthocephalan parasite
763 *Polymorphus minutus* modify the energy reserves and antitoxic defences of its intermediate
764 host *Gammarus roeseli*? *Parasitology* 139(8), 1054–1061.

765 Grabner DS, 2017. Hidden diversity: parasites of stream arthropods. *Freshwater Biology* 62
766 (1), 52-64.

767 González AL, Romero GQ & Srivastava DS, 2014. Detrital nutrient content determines
768 growth rate and elemental composition of bromeliad-dwelling insects. *Freshwater biology*
769 59(4), 737-747.

770 Hladyz, S, Åbjörnsson, K, Chauvet, E, Dobson, M, Elozegi, A, Ferreira, V, Fleituch, T,
771 Gessner, MO, Giller, PS, Gulis, V, Hutton, SA, Lacoursière, JO, Lamothe, S, Lecerf, A,
772 Malmqvist, B, McKie, BG, Nistorescu, M, Preda, E, Riipinen, MP, Rîşnoveanu, G,
773 Schindler, M, Tiegs, SD, Vought, LBM, Woodward, G, 2011. Stream ecosystem
774 functioning in an agricultural landscape: the importance of terrestrial-aquatic linkages.
775 *Advances in Ecological Research* 44(3), 211-276.

776 Holmes JC & Bethel WM, 1972. Modification of intermediate host behaviour by parasites. In:
777 Canning EU, Wright CA (Eds) Behavioural aspects of parasite transmission. Academic
778 Press, London, pp 123-149.

779 Johnson PT, Townsend AR, Cleveland CC, Glibert PM, Howarth RW, McKenzie VJ,
780 Rejmankova E, & Ward MH, 2010. Linking environmental nutrient enrichment and
781 disease emergence in humans and wildlife. *Ecological Applications* 20(1), 16-29.

782 Kennedy C.R., 2006. Ecology of the Acanthocephala. Cambridge University Press,
783 Cambridge.

784 Kraufvelin P, Salovius S, Christie H, Moy FE, Karez R & Pedersen MF, 2006.
785 Eutrophication-induced changes in benthic algae affect the behaviour and fitness of the
786 marine amphipod *Gammarus locusta*. *Aquatic Botany* 84, 199–209.

787 Kuris AM, Hechinger RF, Shaw JC, Whitney KL, Aguirre-Macedo L, Boch CA, Dobson AP,
788 Dunham EJ, Fredensborg BL, Huspen TC, Lorda J, Mababa L, Mancini F, Mora AB,
789 Pickering M, Talhouk NL, Torchin ME & Lafferty KD, 2008. Ecosystem energetic
790 implications of parasite and free-living biomass in three estuaries. *Nature* 454 (7203), 515-
791 518.

792 Labaude, S., Rigaud, T., & Cézilly, F., 2015a. Host manipulation in the face of environmental
793 changes: Ecological consequences. *International Journal for Parasitology: Parasites and*
794 *Wildlife*, 4(3), 442-451.

795 Labaude S, Cézilly F, Tercier X & Rigaud T, 2015b. Influence of host nutritional condition
796 on post-infection traits in the association between the manipulative acanthocephalan
797 *Pomphorhynchus laevis* and the amphipod *Gammarus pulex*. *Parasites & vectors* 8(1), 403.

798 Labaude S, 2016. Effect of the environment on the interaction between gammarids (Crustacea
799 : Amphipoda) and their manipulative acanthocephalan parasites. Thesis of the University
800 of Burgundy, 235pp.

801 Labaude S, Rigaud T & Cézilly F, 2017a. Additive effects of temperature and infection with
802 an acanthocephalan parasite on the shredding activity of *Gammarus fossarum* (Crustacea:
803 Amphipoda): the importance of aggregative behavior. *Global change biology* 23(4), 1415-
804 1424.

805 Labaude S, Cézilly F, Tercier X & Rigaud T, 2017b. Temperature-related intraspecific
806 variability in the behavioral manipulation of acanthocephalan parasites on their gammarid
807 hosts. *Biological Bulletin* 232(2), 82-90.

808 Lafferty KD, Allesina S, Arim M, Briggs CJ, De Leo G, Dobson, AP, Dunne JA, Johnson P,
809 Kuris A, Marcogliese DJ, Martinez N, Memmott J, Marquet PA, McLaughlin J, Mordecai
810 EA, Pascual M, Poulin R, Thielges D, 2008. Parasites in food webs: The ultimate missing
811 links. *Ecology Letters* 11, 533-546.

812 Lagrue, C, Kaldonski, N, Perrot-Minnot, MJ, Motreuil, S & Bollache, L, 2007. Modification
813 of the hosts' behavior by a parasite: field evidence for adaptive manipulation. *Ecology*
814 88(11), 2839-2847.

815 Leflaive J, Felten V, Ferriol J, Lamy A, Ten-Hage L, Bec A & Danger M, 2015. Community
816 structure and nutrient level control the tolerance of autotrophic biofilm to silver
817 contamination. *Environmental Science and Pollution Research* 18, 13739-13752.

818 Lellis WA, Barrows FT & Hardy RW, 2004. Effects of phase-feeding dietary phosphorus on
819 survival, growth, and processing characteristics of rainbow trout *Oncorhynchus mykiss*.
820 *Aquaculture* 242, 607-616.

821 Lettini SE & Sukhdeo MVK, 2010. The energetic cost of parasitism in isopods. *Ecoscience*
822 17, 1-8.

823 Makino W, Urabe J, Elser JJ & Yoshimizu C, 2002. Evidence of phosphorus-limited
824 individual and population growth of *Daphnia* in a Canadian Shield lake. *Oikos* 96, 197-
825 205.

826 Maltby L, Clayton SA, Wood RM & McLoughlin N, 2002. Evaluation of the *Gammarus*
827 *pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness,
828 and relevance. *Environmental Toxicology and Chemistry* 21(2), 361-368.

829 Marcarelli AM, Baxter CV, Mineau MM & Hall RO, 2011. Quantity and quality: unifying
830 food web and ecosystem perspectives on the role of resource subsidies in freshwaters.
831 *Ecology* 92(6), 1215-1225.

832 Marcogliese DJ & Pietrock M, 2011. Combined effects of parasites and contaminants on
833 animal health: parasites do matter. *Trends in Parasitology* 27(3), 123-130.

834 Masclaux H, Bec A, Kainz M, Desvillettes C, Jouve L & Bourdier G, 2009. Combined effects
835 of food quality and temperature on somatic growth and reproduction of two freshwater
836 cladocerans. *Limnology and Oceanography* 54, 1323-1332.

837 Maure F, Brodeur J, Hughes DP & Thomas F, 2013. How much energy should manipulative
838 parasites leave to their hosts to ensure altered behaviours? *The Journal of Experimental*
839 *Biology*, 216, 43–46.

840 Moore J, 2002. Parasites and host behaviour. *Trends in Ecology & Evolution* 17, 585–586.

841 Moore AA & Palmer MA, 2005. Invertebrate biodiversity in agricultural and urban headwater
842 streams: implications for conservation and management. *Ecological Applications* 15(4),
843 1169-1177.

844 Muller-Navarra DC, Brett MT, Park S & Chandra S, 2004. Unsaturated fatty acid content in
845 seston and tropho-dynamic coupling in lakes. *Nature* 427(6969), 69.

846 Naiman RJ, Decamps H, & McClain ME, 2010. *Riparia: ecology, conservation, and*
847 *management of streamside communities*. Elsevier Academic Press, London, 2005, 448pp.

848 Perrot-Minnot M-J, Sanchez-Thirion K, Cézilly F, 2014. Multidimensionality in host
849 manipulation mimicked by serotonin injection. *Proceedings of the Royal Society B:*
850 *Biological Sciences* 281, 20141915.

851 Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Marti E,
852 Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK,
853 Gregory S & Morrall DD, 2001. Control of nitrogen export from watersheds by headwater
854 streams. *Science* 292 (5514), 86-90.

855 Plaistow SJ, Troussard J-P & Cézilly F, 2001. The effect of the acanthocephalan parasite
856 *Pomphorhynchus laevis* on the lipid and glycogen content of its intermediate host
857 *Gammarus pulex*. *International Journal for Parasitology* 31, 346–351.

858 Power ME, 1992. Top-down and bottom-up forces in food webs: do plants have primacy.
859 *Ecology* 73(3), 733-746.

860 Prater, C., Wagner N.D. & Frost, P, 2016. Effects of calcium and phosphorus limitation on
861 the nutritional ecophysiology of *Daphnia*. *Limnology and Oceanography* 61, 268-278.

862 Rollin M, Coulaud R, Danger M, Sohm B, Flayac J, Bec A, Chaumot A, Geffard O & Felten
863 V, 2018. Additive effect of calcium depletion and low resource quality on *Gammarus*
864 *fossarum* (Crustacea, Amphipoda) life history traits. *Environmental Science and Pollution*
865 *Research* 25, 11264-11280.

866 Schade J, Kyle M, Hobbie S, Fagan W & Elser JJ, 2003. Stoichiometric tracking of soil
867 nutrients by a desert insect herbivore. *Ecology Letters* 6, 91-101.

868 Schlotz N, Sørensen JG & Martin-Creuzburg D, 2012. The potential of dietary
869 polyunsaturated fatty acids to modulate eicosanoid synthesis and reproduction in *Daphnia*
870 *magna*: A gene expression approach. *Comparative Biochemistry and Physiology A* 162,
871 449–454.

872 Schlotz N, Ebert D & Martin-Creuzburg D, 2013. Dietary supply with polyunsaturated fatty
873 acids and resulting maternal effects influence host-parasite interactions. *BMC Ecology*
874 13:41.

875 Sims JT, Simard RR & Joern BC, 1998. Phosphorus loss in agricultural drainage: Historical
876 perspective and current research. *Journal of environmental quality* 27(2), 277-293.

- 877 Sornom P, Felten V, Médoc V, Sroda S, Rousselle P & Beisel J-N, 2010. Effect of gender on
878 physiological and behavioural responses of *Gammarus roeseli* (Crustacea Amphipoda) to
879 salinity and temperature. *Environmental Pollution* 158, 1288–1295.
- 880 Stanley-Samuelson DW, 2006. Prostaglandins and other eicosanoids in insects: biological
881 significance. *Annual Review of Entomology* 51 (1), 25–44.
- 882 Sterner RW & Elser JJ, 2002. *Ecological Stoichiometry: the biology of elements from*
883 *molecules to the biosphere*. Princeton University Press, Princeton, USA.
- 884 Stephens JP, Altman KA, Berven KA, Tiegs SD & Raffel TR, 2017. Bottom-up and
885 trait-mediated effects of resource quality on amphibian parasitism. *Journal of Animal*
886 *Ecology* 86(2), 305-315.
- 887 Tain, L, Perrot-Minnot, M-J & Cézilly, F, 2006. Altered host behaviour and brain
888 serotonergic activity caused by acanthocephalans: evidence for specificity. *Proceedings of*
889 *the Royal Society B: Biological Sciences* 273, 3039–3045.
- 890 Vannote RL, Minshall GW, Cummins KW, Sedell JR & Gushing E, 1980. The river
891 continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37, 130-137.
- 892 Wright AN & Gompper ME, 2005. Altered parasite assemblages in raccoons in response to
893 manipulated resource availability. *Oecologia* 144(1), 148-156.