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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  $\mu\text{eq L}^{-1}$ ,  $\text{Cl}^{-}$  = 13.3  $\text{mg L}^{-1}$ ,  $\text{NO}_3^{-}$  = 24.4  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  = 31.7  $\text{mg L}^{-1}$ ,  
179  $\text{PO}_4^{3-}$  < 0.1  $\text{mg L}^{-1}$ ,  $\text{Na}^{+}$  = 6.5  $\text{mg L}^{-1}$ ,  $\text{Ca}^{2+}$  = 100  $\text{mg L}^{-1}$ ,  $\text{K}^{+}$  = 0.82  $\text{mg L}^{-1}$ , and  $\text{Mg}^{2+}$  = 5.76  
180  $\text{mg L}^{-1}$ .

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  $\mu\text{m}$ ; 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  $\text{KH}_2\text{PO}_4$  at 8.71 g phosphorus.L<sup>-1</sup>, 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 mm<sup>3</sup>). 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 HNO<sub>3</sub>, 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 Technologies™ 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:  $10.39 \pm 0.11$  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 cm<sup>2</sup> 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  $15 \text{ mm.s}^{-1}$ , 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  $0.36 \pm 0.08$  mg phosphorus.g<sup>-1</sup> and  
425 manipulated leaves (P) contained  $1.24 \pm 0.16$  mg phosphorus.g<sup>-1</sup>. The dominant fatty acids in  
426 conditioned leaves (S or P) were palmitic acid (16:0), oleic acid (18:1 $\omega$ 9), linoleic acid  
427 (18:2 $\omega$ 6), and  $\alpha$ -linolenic acid (18:3 $\omega$ 3), whereas long chain PUFAs (number of Cs  $\geq$  20)  
428 were totally lacking from these resources.

429 Agarose pellets contained  $1.95 \pm 0.58$  mg phosphorus.g<sup>-1</sup>. In diatoms the major fatty  
430 acids were palmitic acid (16:0), palmitoleic acid (16:1 $\omega$ 7), and EPA (20:5 $\omega$ 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 $\omega$ 7 = 21%, and 20:5 $\omega$ 3 = 16.9%). Docosahexaenoic acid (22:6 $\omega$ 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 =  $4.13 \times 10^{-6}$ , 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).

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