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## Identifying a key host in an acanthocephalan-amphipod system.

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1 **Title page**

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3 Identifying a key host in an acanthocephalan-amphipod system

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6

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## 12 Summary

13

14 Trophically transmitted parasites may use multiple intermediate hosts, some of which may be 'key-hosts',  
15 i.e. contributing significantly more to the completion of the parasite life cycle, while others may be 'sink  
16 hosts' with a poor contribution to parasite transmission. *Gammarus fossarum* and *Gammarus roeseli* are  
17 sympatric crustaceans used as intermediate hosts by the acanthocephalan *Pomphorhynchus laevis*.  
18 *Gammarus roeseli* suffers higher field prevalence and is less sensitive to parasite behavioural manipulation  
19 and to predation by definitive hosts. However, no data are available on between-host differences in  
20 susceptibility to *P. laevis* infection, making it difficult to untangle the relative contributions of these hosts to  
21 parasite transmission. Based on results from estimates of prevalence in gammarids exposed or protected  
22 from predation and laboratory infections, *G. fossarum* specimens were found to be more susceptible to *P.*  
23 *laevis* infection. As it is more susceptible to both parasite infection and manipulation, *G. fossarum* is  
24 therefore a key host for *P. laevis* transmission.

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## 28 Key words

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30 multi-host parasites; prevalence; host specificity; host quality; transmission; infectivity

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32 **Key findings**

33

34 The manipulative *Pomphorhynchus laevis* has both *Gammarus roeseli* and *G. fossarum* as intermediate  
35 hosts.

36 Infected *G. fossarum* are relatively more predated, explaining higher field prevalence in *G. roeseli*.

37 However, differences in susceptibility to *P. laevis* infection between the two host species are unknown.

38 Field experiments and laboratory infections indicate that *G. roeseli* is less susceptible to infection.

39 *Gammarus fossarum* is confirmed as a key host for *P. laevis*.

40

## 41 Introduction

42

43 While the majority of parasites are known to exploit multiple host species, either sequentially or because  
44 they have a range of suitable hosts for the same stage of their cycle (Ruiz-González *et al.*, 2012), host-  
45 parasite interactions are usually studied in simplified one-to-one relations, disconnected from the real-life  
46 complex systems (Rigaud *et al.*, 2010). Multi-host parasites may use host species differing in abundance,  
47 exposure and susceptibility, and thus unlikely to contribute equally to parasite transmission and fitness.  
48 The 'key hosts' are those contributing significantly more to the completion of the parasite life cycle  
49 (Streicker *et al.*, 2013). Three non-exclusive processes serve to identify a host as a key species, contributing  
50 disproportionately to parasite transmission: high host abundance, high exposure and/or susceptibility to  
51 infection, and/or large number of infective stages produced per infected individual (Streicker *et al.*, 2013).

52 Parasites with complex life cycles are, by definition, multi-host parasites because they require at least two  
53 successive host species to achieve their development. However, they may also use several different host  
54 species at any stage of their cycle. Such parasites may show weak specificity when infecting the  
55 intermediate host, or sometimes even the definitive host, although there is great interspecific variation in  
56 these traits (Combes, 2001). Numerous parasites with a complex life cycle have evolved the ability to  
57 modify several aspects of the phenotype of their intermediate hosts, concomitantly increasing the  
58 probability of transmission to their definitive hosts (reviewed in Poulin, 2010). Many trophically transmitted  
59 parasites can even modify certain behaviours of their intermediate hosts (Thomas *et al.*, 2005; Perrot-  
60 Minnot *et al.*, 2014). Modification of a number of anti-predatory behaviours is directly linked to the  
61 modulation of predation rates in intermediate hosts, either increasing for infected versus non-infected  
62 hosts (Kaldonski *et al.*, 2007; Lagrue *et al.*, 2007), or decreasing when the parasites are not yet infective for  
63 the definitive host (Dianne *et al.*, 2011; Weinreich *et al.*, 2013). These behavioural changes have been  
64 referred to as 'host manipulation' because parasites alter the phenotype of their hosts in ways that  
65 enhance their own fitness at the expense of that of infected hosts (Thomas *et al.*, 2005; Cézilly *et al.*, 2010).  
66 For these parasites, the sensitivity of the host to manipulation should be included to determine key host  
67 species, because of its implication in parasite transmission.

68 Acanthocephala are trophically transmitted parasites for which the ability to modify host phenotype is  
69 ubiquitous, possibly having evolved in the common ancestor of the group (Moore, 1984). They all use at  
70 least two hosts to complete their cycle, whether for intermediate, definitive or paratenic hosts, with  
71 different degrees of fitness depending on the hosts and/or spatial distribution of these hosts (see Kennedy  
72 2006 for an overview). *Pomphorhynchus laevis* have been extensively studied in the contexts of host  
73 manipulation and ecology (Kennedy, 2006). They infect several freshwater gammarid amphipod species as  
74 intermediate hosts, and several freshwater fish species as definitive or paratenic hosts (Kennedy, 2006;  
75 Médoc *et al.*, 2011). In central and eastern France, the cryptic *Gammarus pulex* and *Gammarus fossarum*  
76 species (Lagrue *et al.*, 2014) are resident intermediate host species, while *Gammarus roeseli* is a relatively

77 recent colonizer from Southern Central Europe (Jazdzewski, 1980). These gammarids are often found in  
78 sympatry (Chovet and Lécureuil, 1994) and infected by *P. laevis* in these sympatric sites (e.g. Bauer *et al.*,  
79 2000; Rigaud and Moret, 2003; Lagrue *et al.*, 2007). Prevalence and infection intensity are usually higher in  
80 *G. roeseli* than in *G. pulex* (Lagrue *et al.*, 2007, Lagrue, unpublished data), despite the fact that the latter is  
81 generally more abundant than the former when present in sympatry (e.g. Lagrue *et al.*, 2007). It would  
82 therefore seem logical for *P. laevis* to rely more on *G. roeseli* than on *G. pulex* for its transmission. However,  
83 several elements indicate that exactly the opposite situation could be the rule. Crude prevalence is not an  
84 accurate measure to quantify the abundance of a manipulative parasite, since observed prevalence  
85 diminishes as infected intermediate hosts are preferentially preyed upon by the next host(s), rather than  
86 uninfected hosts (Lafferty, 1992; Rousset *et al.*, 1996). Lagrue *et al.* (2007) showed that the prevalence of *P.*  
87 *laevis* in *G. pulex* was low in the river benthos but high in the definitive host's stomach, whereas prevalence  
88 in *G. roeseli* was higher in the field and lower in the stomach of the definitive host. In addition, by analysing  
89 the distribution of parasite intensity, they showed that parasites accumulate in older *G. roeseli*, but not in  
90 older *G. pulex*, confirming a higher death rate of infected *G. pulex* compared to infected *G. roeseli*. This  
91 result is consistent with the fact that infected *G. roeseli* is known to be less strongly manipulated than *G.*  
92 *pulex* by *P. laevis* (Bauer *et al.*, 2000). Furthermore, uninfected *G. roeseli* has been found to be less sensitive  
93 to predation by trout (Bollache *et al.*, 2006) or bullhead (Kaldonski *et al.*, 2008) than uninfected *G. pulex*,  
94 because of more efficient anti-predatory defences. The combination of all these factors provides  
95 reasonable evidence of a predation differential between infected animals of each species, and so *G. roeseli*  
96 can reasonably be considered a lower quality host for *P. laevis* transmission.

97 However, the relative susceptibility of the two amphipod species to infection by *P. laevis* remains  
98 undetermined. Yet this information is crucial to assess the relative importance of the two concurrent hosts  
99 in the *P. laevis* life cycle. If *G. pulex* is more susceptible to infection than *G. roeseli*, then both susceptibility  
100 and behavioural manipulation would act in synergy, making this host a true key host for transmission. If,  
101 conversely, *G. roeseli* is more susceptible than *G. pulex*, then *P. laevis* transmission would be 'diluted' by  
102 the presence of this host, because of its inefficiency in transmitting the parasite, and could potentially  
103 impact the epidemiology of the infection (see Hall *et al.*, 2009; Johnson *et al.*, 2009, for examples). We  
104 conducted a laboratory infection experiment by submitting both species to the same dose of *P. laevis* eggs  
105 to measure the susceptibility of these sympatric gammarid species to *P. laevis*. To assess the impact of  
106 predation, we compared prevalence in two contrasted amphipod collections from the field: animals directly  
107 collected from rivers (i.e. previously exposed to natural predation), and animals collected from the same  
108 rivers, but then maintained for several weeks in the laboratory (i.e. in the absence of any fish predation  
109 pressure).

## 110 **Methods**

### 111 ***Amphipod collection and prevalence in the field***

112 Since field prevalence may be variable between populations, two rivers were chosen, where *G. fossarum*  
113 and *G. roeseli* live in sympatry and are naturally infected by *P. laevis*. Amphipods from the Albane River, in  
114 Trochères (47°20'34"N, 5°18'21.8"E), and the Meuzin River, near Villy-le-Moutier (47°2'7.71"N,  
115 4°59'53.87"E), were sampled between September and October 2013.

116 Amphipods (*G. roeseli* and *G. fossarum*) were captured using kick nets. All potential habitats present at  
117 each site were sampled, and the collected animals were randomly divided into three groups, each  
118 maintained in a container with aerated water from the river.

119 The first group was used to estimate the 'field/direct' prevalence. Animals from this group were kept in  
120 well aerated aquaria at 15 °C and all checked for parasite presence within two days after capture. Infected  
121 individuals were dissected to confirm parasite species. Larval parasites can be detected through the host  
122 cuticle, either at the late acanthella stage of their development (translucent light orange, shapeless larval  
123 stage) or at cystacanth stage (bright yellow-orange, spherical larval stage). Earlier acanthella stages (where  
124 parasites are small and translucent) can only be detected after dissection. Preliminary investigation showed  
125 that acanthella detection could only be certified after 40 days (without microscope and staining), so that all  
126 prevalence reported in the following experiments is prevalence for *P. laevis* of more than 40 days old  
127 (Labaude et al., submitted).

128 Gammarids from the second group were kept individually in the laboratory, in cups of c.a. 50 mL at 15 °C  
129 for 96 days. All gammarids where infection was detectable by eye were removed from this group so that, at  
130 the beginning of this survey, the remaining animals were classified as "uninfected". However, as previously  
131 stated, younger acanthella stages are too small to be detected through host cuticle, so some of these  
132 isolated gammarids may have already been infected in the field in the days preceding their capture. It is the  
133 prevalence of these undetected infections that was recorded during this survey. Animals dying during this  
134 period were dissected the day after their death, and all living animals were checked and dissected 96 days  
135 post isolation, a delay long enough to ensure that all parasites could be detected. This survey therefore  
136 allowed prevalence to be estimated in gammarids not exposed to predation during parasite development  
137 (hereafter called 'field/protected' prevalence). All infected *G. fossarum* were kept in ethanol for genetic  
138 analysis (see above).

139 A third group of gammarids was used for experimental infections (see below).

### 140 ***Experimental infection***

141 Before being isolated for the experiment, all gammarids were inspected under a dissecting microscope to  
142 remove naturally infected animals. The remaining gammarids were kept in quarantine for 30 days, to

143 distinguish any further natural infection (by parasites too young to be detected) from experimental  
144 infection. Some additional *G. pulex* were also collected in a small tributary of the Suzon River at Val-Suzon  
145 (47°4'12.6"N; 4°52'58.2"E). Given that the *G. pulex* from Val-Suzon are particularly sensitive to  
146 experimental infection by *P. laevis* (Franceschi *et al.*, 2010), they were used to confirm the success and  
147 timing of experimental infection.

148 Gravid *P. laevis* females were collected from the intestines of chubs (*Leuciscus cephalus*), from naturally  
149 infected fish caught in September 2013 in the Vouge River (Burgundy, Eastern France: 47°9'34.36"N;  
150 5°9'2.50"E). A foreign parasite population was chosen to avoid potential local adaptation in our two  
151 gammarid populations (Franceschi *et al.*, 2010), so that it was possible to estimate gammarid sensitivity to  
152 parasite strains with which they had not evolved. Molecular identification of parasites and exposure of  
153 gammarids to parasite eggs followed the procedure described in Franceschi *et al.* (2008). *Gammarus*, in  
154 cups filled with c.a. 50 mL of aerated water, were allowed to feed for 48 hours on a 1 cm<sup>2</sup> piece of elm leaf,  
155 on which a suspension of 100 mature eggs per gammarid had been deposited (see detailed procedure in  
156 Franceschi *et al.*, 2008). Food was then removed, and gammarids were maintained at 15 °C for 3 months.  
157 The field/protected group described above was used as control. Individuals from this group were treated  
158 and maintained under the same conditions as exposed gammarids but were unexposed to parasite eggs. A  
159 total of 615 *G. fossarum* (162 males and 109 females from Albane, 214 males and 130 females from  
160 Meuzin) and 440 *G. roeseli* (157 males and 102 females from Albane, 121 males and 60 females from  
161 Meuzin) were exposed to parasite eggs, as were the *G. pulex* (155 males from Val-Suzon). 308 *G. fossarum*  
162 (104 males and 61 females from Albane, 89 males and 54 females from Meuzin) and 324 *G. roeseli* (102  
163 males and 67 females from Albane, 104 males and 51 females from Meuzin) were used as control  
164 individuals. All infected *G. fossarum*, along with 100 individuals from the control group, were kept in  
165 ethanol for genetic investigation (see below).

166 The water of each dish was completely renewed every two weeks with aerated water from the river, and  
167 water levels were restored to original levels twice a week. The amphipods were fed *ad libitum* with elm  
168 leaves, and their diet was enriched with a chironomid larva twice a month. A daily mortality survey was  
169 carried out, and animals were dissected the day after their death to detect young acanthella stages. From  
170 the sixth week post-exposure, living gammarids were inspected every week under a dissecting microscope  
171 to detect the presence of parasites. Infected animals were examined every two days after detection to  
172 estimate the date when the cystacanth stage was reached. Gammarids from Val-Suzon (where *P. laevis* is  
173 absent) were a control group for the timing and success of experimental infection. Previous studies  
174 revealed that *P. laevis* reaches cystacanth stage in about 80-120 days in laboratory conditions (Franceschi  
175 *et al.*, 2008, 2010). In gammarids from the Meuzin and Albane rivers, even after a quarantine of 30 days  
176 before exposure, parasites from the wild can develop. Therefore, if *P. laevis* were detected before the first  
177 signs of infection in animals from Val-Suzon, individuals were removed from the analysis to avoid any  
178 potential confounding effect.



## 179 ***Gammarid genotyping***

180 Because of the recently discovered cryptic genetic diversity within the *Gammarus fossarum-pulex* species  
181 complexes (e.g. Lagrue *et al.*, 2014), there is a need to examine patterns of infection in the light of this  
182 diversity (see Westram *et al.*, 2011). Such a study is not necessary for *G. roeseli* because no cryptic diversity  
183 has been detected in Western and Central Europe (Moret *et al.*, 2007). Genetic diversity was assessed in  
184 these two rivers using the amplification of part of the mtDNA cytochrome c oxidase subunit 1 (CO1) by  
185 polymerase chain reactions (PCR) and a subsequent RFLP procedure (Lagrue *et al.*, 2014). Only *G. fossarum*  
186 belonging to one group were known to occur at the Meuzin site (GfI, see Lagrue *et al.*, 2014), while genetic  
187 diversity for the Albane River had not previously been estimated. All infected *G. fossarum* and *G. pulex*  
188 from each river were preserved in pure ethanol after death, for subsequent DNA extraction. In addition,  
189 100 uninfected animals randomly sampled from each site were also preserved. Gammarid DNA was  
190 extracted from two pereopods ('walking legs' in amphipods), following the standard chelex method (Lagrue  
191 *et al.*, 2014). The DNA was then amplified for CO1 using universal primers (LCO1490 and HCO2198; Folmer  
192 *et al.*, 1994). The PCR were performed using Qiagen Multiplex DNA polymerase kits (Qiagen Inc, Düsseldorf,  
193 Germany), as in Lagrue *et al.* (2014). The PCR-amplified DNA products were then digested overnight using  
194 the appropriate reaction buffer and restriction endonuclease(s), following manufacturer's instructions  
195 (New England Biolabs, Ipswich, Massachusetts, USA). The resulting fragments were separated by gel  
196 electrophoresis in a 1.5 % agarose gel. Restriction enzyme profiles were used to assign each individual  
197 amphipod to its respective genetic group (see Lagrue *et al.*, (2014) for the detailed procedure and the  
198 specific digestion enzymes for each gammarid genetic group).

199

## 200 ***Statistical analyses***

201 All statistical analyses were performed using R software (R Development Core Team, 2013) or JMP software  
202 (version 10.0.0).

203 For natural infections, a binomial logistic regression was performed to analyse prevalence, with the  
204 following potential explanatory factors: site (Albane River vs. Meuzin River), *Gammarus* species (*G. roeseli*  
205 vs. *G. fossarum*), *Gammarus* sex (males vs. females), experiment (field/direct: natural infection from the  
206 field sample vs. field/protected: natural infection after maintenance in the laboratory), and their second-  
207 order interactions.

208 For experimental infections, a binomial logistic regression was performed to analyse prevalence, with site,  
209 species and sex, and their second-order interactions, as potential explanatory factors.

210 All possible models were compared using the Akaike Information Criterion (AICc). The models presented  
211 are those minimizing the AICc.

212

## 213 **Results**

### 214 ***Genetic diversity among G. fossarum-like gammarids***

215 For the gammarids from the Albane River, PCR-RFLP revealed 87 % of *G. fossarum* and 13 % of *G. pulex* in  
216 the 50 randomly sampled, uninfected animals, with 82 % of *G. fossarum* and 18 % *G. pulex* in the 68  
217 infected animals. The species ratios in infected and uninfected groups were not significantly different ( $\chi^2 =$   
218 0.2438,  $P = 0.6215$ ). As we detected no difference in sensitivity to infection between *G. pulex* and *G.*  
219 *fossarum*, and since the majority of the gammarids, even at the Albane site, are *G. fossarum*, this term is  
220 used to encompass all *G. fossarum*-like gammarids.

221

### 222 ***Natural infection: direct field prevalence vs. field prevalence protected from***

#### 223 ***predation***

224 Prevalence of *P. laevis* was higher in *G. roeseli* than in *G. fossarum* in direct field prevalence, at both sites,  
225 whereas reverse relative prevalence was observed when measured after keeping putative uninfected  
226 animals in the laboratory, where they were preserved from predation (Table 1, Figure 1).

227

### 228 ***Experimental infection***

229 The first observations of acanthellae through the host cuticle occurred 60 days post-exposure for the  
230 control Val-Suzon gammarids, as was the case for gammarids of both species from the Albane and Meuzin  
231 rivers. The cystacanth stage was achieved  $82 \pm 10$  days post-exposure of the control Val-Suzon group, after  
232  $80 \pm 6$  days for *G. fossarum*, and after  $83 \pm 3$  days for *G. roeseli*.

233 We found a strong effect of river origin on infection (Table 2, Figure 1), with gammarids from the Albane  
234 River being three times more sensitive to infection. The difference in prevalence between species, with *G.*  
235 *fossarum* being approximately twice as infected as *G. roeseli*, was nevertheless not strong enough to be  
236 fully supported statistically (Table 2, Figure 1).

237

## 238 Discussion

239

240 Our data initially showed that the crude *P. laevis* prevalence is higher in *G. roeseli* than in *G. fossarum*,  
241 confirming results of Lagrue *et al.* (2007) for another site. In the 'field/protected' experiment, the  
242 prevalence was reversed, and was higher in *G. fossarum* for the two populations investigated. In addition,  
243 prevalence in *G. fossarum* was approximately twice that in *G. roeseli* in both populations after  
244 experimental infection by a non-coevolved parasite population, even though this result was not fully  
245 supported statistically (probably due to the stronger population effect). Prevalence observed in the field is  
246 therefore not a reliable measure of the actual parasite burden for this manipulative trophically transmitted  
247 parasite. Differences in the duration of parasite development could possibly have explained the differences  
248 in prevalence observed between the two *Gammarus* species. However, parasite growth was synchronous  
249 for all hosts during the laboratory infection experiment.

250

251 As the two hosts have similar lifespans, parasites developing in *G. roeseli* have a lower probability of  
252 completing their life cycle, due both to reduced natural predation by fish compared to *G. pulex* (Bollache *et al.*, 2006; Kaldonski *et al.*, 2008) and lower manipulation levels for infected individuals (Bauer *et al.*, 2000).  
253 Therefore, *G. roeseli* seems to 'dilute' *P. laevis* transmission when this host is sympatric with *G. fossarum*.  
254 However, as shown here, *G. roeseli* is not more susceptible than *G. fossarum* to infection by *P. laevis*, so the  
255 dilution effect is not as strong as previously thought when natural prevalence alone was considered. Lower  
256 infection success in *G. roeseli* counterbalances the low predation rate, limiting the 'sink effect' for the  
257 parasite. As *G. fossarum* is first more susceptible to infection and then more predated, our data confirm  
258 this species as a key host for *P. laevis*.

260

261 Our results also have implications in explaining the role of parasites in the success of biological invasions.  
262 *Gammarus roeseli* is a species that colonised Western Europe during the 20<sup>th</sup> century (Chovet and  
263 Lécureuil, 1994). Parasitism may play a role in the coexistence of native and introduced (or invasive) host  
264 species. Some studies support the 'enemy release' hypothesis, in which invaders are no longer exposed to  
265 their original parasites, but also less susceptible to infection by native parasites, providing invasive hosts  
266 with a competitive advantage (Dunn and Dick, 1998; Kopp and Jokela, 2007). In contrast, other studies  
267 show a decrease in prevalence in native species by the dilution effect, both experimentally (Kopp and  
268 Jokela, 2007) and *in natura* (Telfer *et al.*, 2005). The invader acts in that case as a dead-end sink for the  
269 parasite. *G. roeseli* being less susceptible to both infection (this study) and to behavioural changes induced  
270 by *P. laevis* (Bauer *et al.*, 2000; Moret *et al.*, 2007), our results are in line with the enemy release  
271 hypothesis.

272

273 Our results complete and strengthen the hypothesis that sympatric *G. roeseli* and *G. fossarum* are not hosts  
274 of the same quality for acanthocephalan parasites. Should this assumption be extended to all gammarid  
275 hosts of freshwater acanthocephalans? Because of the high level of cryptic speciation in the *Gammarus*  
276 *pulex/fossarum* group (e.g. Westram *et al.*, 2011b; Lagrue *et al.*, 2014), the situation will probably be quite  
277 complex to study. Westram *et al.* (2011a), coupling natural prevalence estimations and field infection  
278 experiments, also showed differences in susceptibility between *Gammarus* species to infection by the  
279 acanthocephalan *Pomphorhynchus tereticollis*, with *G. pulex* being less infected than *G. fossarum*.  
280 Differences within the *G. fossarum* group, while less marked, were also detected. However, in Switzerland,  
281 where the study was carried out, different species (and/or cryptic species) are rarely found in sympatry,  
282 each stream or river harbouring a single gammarid species, so there is confusion between host species and  
283 the sites where the host-parasite couple is living, with the potential for local adaptation confounding the  
284 results of host specificity (Franceschi *et al.*, 2010). Apart from our case-study of the *G. roeseli* / *G. fossarum*  
285 system, no clear data are available yet on infectivity and behavioural changes induced by the same local  
286 parasite strains on two sympatric species. In the present study, we found no significant difference in  
287 prevalence between sympatric *G. pulex* and *G. fossarum* from the Albane River. However, this result should  
288 be replicated in other rivers, with more individuals and more species tested. Behavioural modifications  
289 should also be measured to confirm this apparent lack of specificity.

290

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292

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296

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301

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1 Table 1. Logistic regression testing for the effects of site (river), *Gammarus* species and experiment (direct  
 2 field prevalence or protected field prevalence) on the field prevalence of *P. laevis*. The model initially  
 3 included sex of gammarids, and other interactions. After removing these non-significant factors, the model  
 4 presented now minimizes the AICc.

5

Source of variation	d.f.	LR $\chi^2$	P
Site	1	1.2999	0.2542
Species	1	0.8110	0.3678
Experiment	1	0.1877	0.6648
Species*experiment	1	7.7271	0.0054
Site*experiment	1	2.3673	0.1239

6 Global model: LR  $\chi^2 = 15.4448$ , 5 d.f., P = 0.0086; n = 1787



1 Table 2. Logistic regression testing for the effects of site (river) and *Gammarus* species on the prevalence of  
 2 *P. laevis* after experimental infection by parasites from the Ouche River. The model initially included sex of  
 3 gammarids and interactions. After removing these non-significant factors, the model presented now  
 4 minimizes the AICc.

5

Source of variation	d.f.	LR $\chi^2$	P
Site	1	16.9051	< 0.0001
Species	1	3.3303	0.0680

6 Global model: LR  $\chi^2 = 19.9606$ , 2 d.f.,  $P < 0.0001$ ;  $n = 807$

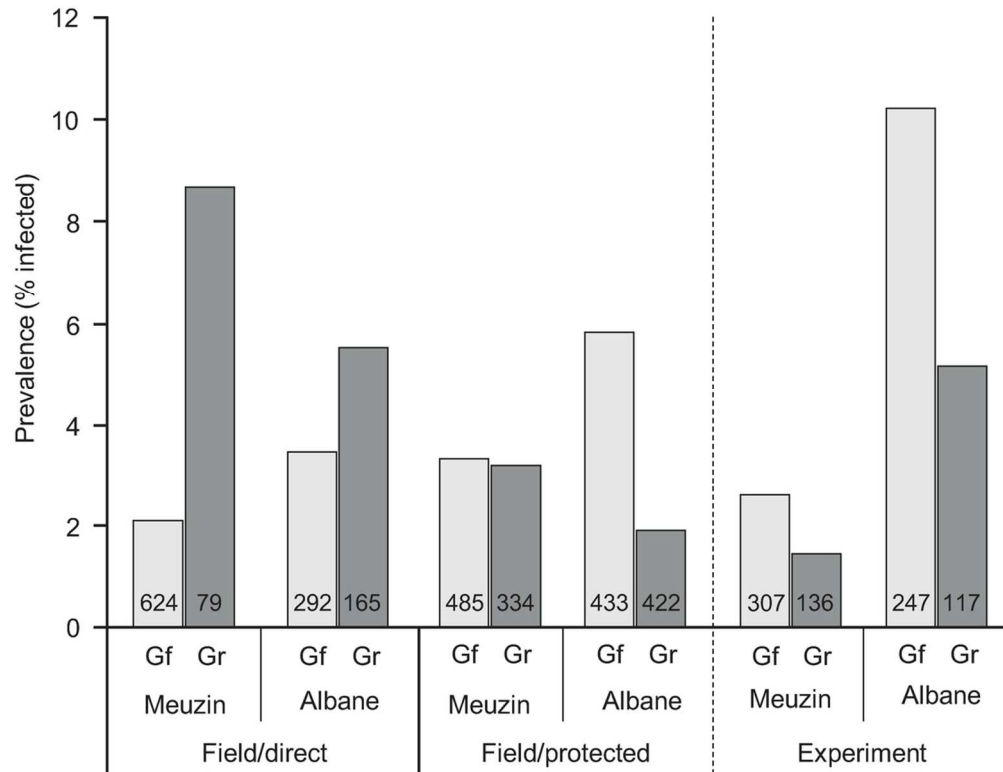


Figure 1: Prevalence levels for *G. fossarum* (Gf) and *G. roeseli* (Gr) in the two populations, for all experiments (field/direct: prevalence in natura; field/protected: prevalence in gammarids kept in the laboratory, i.e. protected from predation; experiment: experimental infection). Number in bars are sample size.

106x81mm (300 x 300 DPI)