

Parasites make hosts more profitable but less available to predators

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Abstract

Parasites are omnipresent, and their eco-evolutionary significance has aroused much interest from scientists. Parasites may affect their hosts in many ways by altering host density, vulnerability to predation, and energy content, thus modifying profitability within the optimal foraging framework. Consequently, parasites could impact predator diet and trophic links through food webs. Here, we investigate the consequences of the iridovirus *Daphnia iridescent virus 1* (DIV-1) infection on the reproductive success, mortality, appearance, mobility, and biochemical composition of water fleas (*Daphnia magna*), a widespread freshwater crustacean. We compare search time between infected and uninfected *Daphnia* preyed by a common aquatic insect (*Notonecta* sp.) as well as the handling time and feeding preference of *Notonecta* sp. Our findings show that infection does not change fecundity but reduces lifespan and thereby constrains fitness. Infected *Daphnia* show reduced mobility and increased color reflectance in the UV and visible domains, which potentially affects their visibility and thus catchability. Infection increases body size and the amount of proteins but does not affect carbohydrate and lipid contents. Although infected *Daphnia* had a longer handling time, they are preferred over uninfected individuals by aquatic insects. Taken together, our findings show that DIV-1 infection could make *Daphnia* more profitable to predators (21% energy increase), a positive effect that should be balanced with density reductions due to higher mortalities. We also highlight that exposure to infection in asymptomatic individuals leads to ecological characteristics that differ from both healthy and symptomatic infected individuals.

Keywords: *Daphnia magna*, white fat cell disease, optimal foraging theory, parasite-induced phenotypic alterations, European minnow, *Notonecta* sp.

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Introduction

48 All living organisms are concerned by parasitism, either as hosts or because they
49 practice this strategy themselves at some point in their lifecycle (Dobson et al., 2008).
50 Infection is generally accompanied by subtle or severe alterations in host phenotypes,
51 including changes to physiology, morphology, and behavior with potential consequences
52 on fitness (Thomas et al., 2010). Host fitness can be impacted directly through reduced
53 fecundity or increased mortality, or indirectly when phenotypic alterations make the
54 hosts more vulnerable to their natural enemies, including predators. Few studies, that
55 work on the diversity of parasite-induced phenotypic alterations, have simultaneously
56 considered both direct and indirect effects (Cézilly et al., 2013). From the predators'
57 perspective, their fitness can also be indirectly affected by prey infection, leading to the
58 possible avoidance of infected prey (Flick et al., 2016).

59 The direct effects of infection result from the rerouting of metabolic energy from the
60 host to parasite growth, maturity, and reproduction, with the intensity depending on
61 parasite virulence. Virulence can be defined as the extent to which a parasite exploits its
62 host and thus reduces its survival and fecundity (Read, 1994). Owing to its importance,
63 virulence is very often assessed in host-parasite interactions (Prins & Weyerhaeuser,
64 1987; Newey & Thirgood, 2004). For instance, some parasites of water fleas (e.g., fungus,
65 bacteria, trematode) reduce egg production and increase mortality (Schwartz & Cameron,
66 1993; Decaestecker et al., 2003). Host survival can also decrease indirectly (i.e., implying
67 a third species) when infected hosts become more vulnerable to predation, which is either
68 considered adaptive from the point of view of the parasite when the predator is the next
69 host (see the manipulation hypothesis, Bethel & Holmes, 1977; Lefèvre et al., 2009;
70 Jacquin et al., 2014), or a simple by-product of infection. For instance, the reduced body
71 condition of infected moose makes them more prone to be eaten by wolves (Peterson &

72 Page, 1988), while infected red goose are more readily attacked by mammalian predators
73 (Hudson et al., 1992). Similarly, infection with the nematode *Gasteromermis* sp. reduces
74 larval drift in the insect *Baetis bicaudatus*, which becomes more vulnerable to predation
75 by the sickle springfly *Kogotus modestus* but not to predation by the caddisfly *Rhyacophila*
76 *hyalinata*, thus suggesting a predator-dependent effect (Vance & Peckarsky, 1997). Host
77 weakening (see the review of Sánchez et al., 2018) may be due to energy reallocation to
78 parasite growth (Hall et al., 2007) or the cost of the immune response (Otti et al., 2012).
79 Increased vulnerability can also result from changes in host appearance (e.g., coloration,
80 size). For instance, *Polycaryum laeve* (Chytridiomycota) infection causes opacification in
81 *Daphnia pulicaria*, which may increase its vulnerability to fish predation (Johnson et al.,
82 2006).

83 Parasite-induced phenotypic alterations in prey are likely to influence the diet of
84 predators. Optimal foraging theory predicts that the inclusion of a particular prey to the
85 diet of a predator depends on its relative abundance and profitability ranking (Emlen,
86 1966; MacArthur & Pianka, 1966; Charnov, 1976a; b). Profitability is the ratio between
87 the energy content of the prey and its handling time for a given search time. By diverting
88 energy, parasites modify the biochemical content of their host. In particular, Plaistow et
89 al. (2001) reported a decrease in glycogen content and an increase in lipid content in
90 crustacean amphipods infected by the acanthocephalan parasite *Pomphorhynchus laevis*.
91 For *Daphnia pulicaria* infected by *Polycaryum laeve*, the increase in carbon content and
92 the reduction in nitrogen and phosphorus increased the carbon-to-nitrogen ratio
93 (Forshay et al., 2008). When energy content is increased by infection, hosts might
94 conversely become more profitable to predators if the handling time remains unchanged.
95 Similar effects are expected when alterations in behavior and aspect make host weaker

96 (reducing prey escape) and more visible, and thus more vulnerable (lower search time
97 and handling time) to predation.

98 To understand the effects of parasitism in a trophic context, it is crucial to study
99 concomitantly the different host alterations and their relative intensity. To address this
100 issue, we used as host species the water flea *Daphnia magna*, a widespread freshwater
101 crustacean that plays a central role in food webs, both as an herbivore and as a prey
102 (Lampert & Sommer, 2007; Reynolds, 2011; Ebert, 2022). *Daphnia magna* can host a
103 diversity of parasites (Green, 1974; Ebert, 2005, 2022), including the *Daphnia* iridescent
104 virus 1 (DIV-1, Toenshoff et al., 2018), which is known to increase mortality and reduce
105 fecundity in infected individuals (Ebert et al., 2000) as well as alter their activity, thus
106 affecting their potential profitability to predators. It also impacts host appearance
107 through the induction of a white phenotype, and consequently, DIV-1 have been known
108 as “White Fat Cell Disease” (WFCDD) but wrongly labeled as “White Bacterial Disease”
109 (WBD). However, information on phenotypic modifications and their implications
110 regarding vulnerability to predation are lacking, which prevents us from fully
111 understanding the consequences of parasitism in an optimal foraging context. We
112 quantified the alterations in terms of fecundity, survival, mobility, coloration, body size,
113 biochemical content (carbohydrates, lipids, and proteins), and vulnerability to predation
114 (by *Notonecta*, a common generalist predator (Giller, 1986; Van der Lee et al., 2021) and
115 fish) using both *in situ* and experimentally infected *D. magna*. Considering previous
116 research on the virulence of DIV-1 (Ebert et al., 2000), we expect high direct effects with
117 a reduction in host survival and fecundity. Indirect effects are studied here for the first
118 time, and we expect the energy costs of infection to reduce host activity, thus favoring
119 predation, which could be further facilitated by the white coloration of infected water
120 fleas.

Material and Methods

Collection and maintenance of organisms

Daphnia magna (identified according to the morphological characteristics described by Amoros, 1984) and the parasite were collected from two ponds in Paris (France): La Villette (48°53'43.0"N 2°23'26.5"E) and Bercy (48°50'03.0"N 2°23'03.1"E) where DIV-1 prevalence ranges from 0.5 to 3% (pers. obs.). Given the high host specificity of DIV-1, collecting hosts and parasites from the same pond was expected to promote the success of the experimental infection (Decaestecker et al., 2003). DIV-1-infected *D. magna* have a highly identifiable phenotype: under light, infected fat cells are blue-white, almost fluorescent (Ebert, 2005).

All *D. magna* individuals were stored in 5 L rearing tanks (100-150 ind.L⁻¹) filled with filtered water from their collection pond. Depending on the experiment, they were used on the day of capture or stored for up to 3 days without food supply at 20 °C. To identify infected individuals and isolate parasites, the crustaceans were placed in a black jar and illuminated to observe any phenotypic signs of infection. Infected and non-infected *D. magna* were kept separately in Volvic® mineral water at 20 °C under a 12:12 light:dark cycle (200 Lux) at the same density of 100 ind.L⁻¹ in 1 L tanks.

Vulnerability to predation was investigated using an aquatic insect from the *Notonecta* genus and a fish, the European minnow *Phoxinus phoxinus* (Appendix A). *Notonecta* sp. (1.8-2.0 cm in total length) were collected from a pond at Orsay (France, 48°42'04.4"N 2°10'42.7"E) using a hand net. Immediately after collection, they were stored and starved in 5 L of water from the pond (3 ind.L⁻¹) for 1 day before the beginning of the experiments.

In this study, we performed an experimental infection to determine the effects of DIV-1 on fecundity (Measure 1), mortality (Measure 2), mobility (Measure 3), and size

Table 1. Summary of measures performed for each collected *D. magna*.

| Pond | Sampling date | Infection | Measure 1 | Measure 2 | Measure 3 | Measure 4 | Measure 5 | Measure 6 | Measures 7/8/9 |
|-------------|---------------|--------------|-----------|-----------|-----------|-----------|-----------|-------------|-------------------------------|
| | | | Fecundity | Mortality | Mobility | Size | Energy | Reflectance | Predation |
| La Villette | 07/2017 | Experimental | X | X | X | X | | | |
| Both | 04-07/2018 | Natural | X | | | X | | | |
| La Villette | 09/2017 | Natural | | | X | | X | | |
| Bercy | 05/2018 | Natural | | | X | | X | | |
| La Villette | 07/2018 | Natural | | | | | | X | |
| Bercy | 04/2018 | Natural | | | | X | | | X (7, Fish) |
| La Villette | 07/2018 | Natural | | | | X | | | X (7,8, 9, <i>Notonecta</i>) |

145 (Measure 4). We also used naturally-infected individuals to measure fecundity
146 (Measure 1), mobility (Measure 3), size (Measure 4), energy content (Measure 5),
147 coloration (Measure 6), vulnerability to predation (Measure 7&8), and predator
148 preference (Measure 9). Table 1 summarizes the measures performed on each collected
149 *Daphnia*.

150 **Fecundity and mortality (Measures 1 and 2)**

151 Reproductive success (Measure 1) and survival (Measure 2) were assessed in two
152 manners: in the laboratory through experimental infections (Measures 1 and 2) and from
153 wild individuals (Measure 1). The experimental infection allowed us to clearly distinguish
154 between the effects on fecundity and survival. We do not consider offspring production
155 along lifetime as a proxy of fecundity, but rather as a proxy of fitness, because it
156 encapsulates both fecundity parameters (clutch size, clutch frequency, and age at
157 maturity) and survival (lifespan).

158 Gravid *D. magna* collected from the La Villette pond in July 2017 and stored in their
159 rearing tanks were transferred individually to 50 mL jars containing Volvic® water.
160 Newborns (<24h) were transferred individually into jars with 45 mL of Volvic® water in
161 a climatic chamber at 20 °C, and fed with 0.25 mL of *Scenedesmus obliquus* (2.3×10^6

162 cells.mL⁻¹) every 3 days throughout the experiment. These algae were obtained from the
163 Muséum National d'Histoire Naturelle (Paris, France, algothèque MNHN; strain number:
164 ALCP n°349), and cultivated at 20 °C under a 12:12 light:dark cycle in an ES medium
165 (Basal Medium, "Erddekot + Salze" described by Culture Collection of Algae of Sammlung
166 von Algenkulturen Göttingen). Molts were removed daily to maintain water clarity.

167 To infect *D. magna*, we prepared a solution of infected *D. magna* cadavers (hereafter,
168 parasite solution) homogenized at the concentration of 1 cadaver/mL in Volvic® water.
169 A control solution was prepared with healthy cadavers. Half of the newborns were
170 exposed to the parasite solution and the other to the control solution. On Day 1, we added
171 1 mL of the solution to obtain a ratio of 1 cadaver per juvenile of *D. magna*. On Days 4 to
172 6, we stirred the water (both the control and treatment) using a pipette to resuspend the
173 spores and promote infection. Water was replaced on Day 15 by clean water (without the
174 virus) and then once a week until the death of the last individual of *D. magna* (163 days).
175 Offspring were removed and counted daily, and dead *D. magna* were controlled visually,
176 as described above, for infection signs. We started two sets of experimental infections
177 with 1 day of delay: the first set was performed with 27 juveniles (14 exposed to the
178 parasite solution and 13 to the control solution) coming from 11 distinct mothers, while
179 the second set was performed with 44 juveniles (23 exposed to the parasite solution and
180 21 to the control solution), also coming from 11 distinct mothers.

181 For naturally-infected individuals, collection took place in April-June 2018 in the two
182 ponds (Bercy and La Villette). We sampled 20 L of water filtered with a 50 µm net to
183 collect *D. magna*. After separating infected and non-infected *D. magna*, individuals were
184 fixed using glycerol solution (1% glycerol, 70% ethanol, 29% water). We then categorized
185 individuals as broodless (without eggs or ephippia), egg-carrying (with parthenogenetic
186 eggs), and ephippia-carrying (with sexual ephippia).

187 **Mobility (Measure 3)**

188 We assessed mobility in two ways: (i) using the experimentally exposed individuals
189 from Measure 1 that were still alive on day 14 (n = 53), and (ii) using naturally exposed
190 individuals collected from the La Villette pond in September 2017 (n = 188) and the Bercy
191 pond in May 2018 (n = 135), stored in rearing tanks and assessed within a day after
192 collection. These naturally infected individuals were subsequently used for Measure 5
193 (see below). We measured speed (maximal and mean), swimming time, and the number
194 of turnings as described by Untersteiner et al. (2003) and Bownik (2017). The water fleas
195 were placed individually into one of the nine chambers (3 x 3.2 x 1 cm, Lxlxh) of a grid in
196 a black box filled with Volvic® water. We placed a light source (150 Lux) under the grid
197 with a video camera (Canon® EOS 70D body with Canon® EF-S 17-55mm f/2.8 IS USM
198 lens) placed 52 cm above. After 5 min of acclimatization, *D. magna* were filmed for 29 sec,
199 divided into five sequences of 3.80 sec, each interrupted by 5 sec intervals between two
200 consecutive sequences, in monochrome at a rate of 25 fps. By making five films per animal,
201 we reduced the risk of misdetection by the software. Several sequences in which *D. magna*
202 were not detected were not analyzed, and mobility was instead evaluated in the three or
203 four remaining films. Video analysis was performed with the ImageJ software (version
204 1.4.3.67) and the plugin wrMTrck (31/10/2011 version by Jesper Søndergaard Pedersen,
205 modified by the authors). We subtracted the background and shifted from grayscale to
206 black and white to promote detection. The plugin allowed us to identify the group of black
207 pixels corresponding to *D. magna* and determine the mobility parameters (mean and
208 maximum speeds, rotating movements). We modified the plugin to assess inactivity time:
209 the absence of movement between two consecutive records was converted in time by
210 considering the time interval between these two sequences (here 1/25 sec).

211 **Body size (Measure 4)**

212 To measure individual size (from the head to the start of the caudal spine) of the
213 experimentally-infected *D. magna* used for Measures 1 & 2, we used the video recordings
214 obtained for the mobility assessment (Measure 3, n = 53 individuals). Body size was
215 measured with a micrometer screw for naturally-infected *D. magna* among those
216 collected in the La Villette and Bercy ponds (Measure 1, n = 435). We also used the
217 photographs of a set of *D. magna* used in the predation experiments (Measure 7, see
218 below, n = 229) to determine their size. Specimens of *D. magna* taken from photographs
219 and videos were measured with ImageJ software (version 1.4.3.67).

220 **Biochemical composition and energy value (Measure 5)**

221 We assessed the quantity of carbohydrates, lipids, and proteins per mg of *D. magna* in
222 the naturally-infected *D. magna* used for Measure 3. For each pond, we considered three
223 categories of crustaceans: broodless individuals (no visible signs of infection, no eggs),
224 brooding individuals (no visible signs of infection, with eggs), and infected individuals
225 (visible signs of DIV-1 infection, without eggs). Unfortunately, we did not collect enough
226 DIV-1 infected *D. magna* with eggs to conduct biochemical assays. Preliminary tests
227 showed that pools of 10 individuals were optimal to obtain a reliable signal for accurately
228 measuring the amount of proteins, sugars, and triglycerides. Immediately after the
229 mobility experiment, groups of 10 *D. magna* individuals were snap-frozen and stored at -
230 25 °C after removing any water with a towel.

231 The concentrations of proteins, sugars, and triglycerides were measured using
232 colorimetric assays, as described by Ouisse et al. (2017) and Foray et al. (2012). Briefly,
233 each pool of 10 crustaceans was first weighed (Fresh mass, Balance XP2U Mettler Toledo,
234 Columbus, OH, d=0.1 µg). After the addition of 200 µL of phosphate buffer (pH 7.2), each
235 pool was homogenized for 90 sec at 25 Hz (bead-beating device, Retsch™ MM301, Retsch
236 GbmH, Haan, Germany). The pools were then centrifuged (180 g, for 10 min, 4 °C), and a

237 volume of 8 μL of supernatant was collected to quantify the amount of proteins using the
238 Bradford method (Bradford, 1976). The absorbance of samples was read at 595 nm, and
239 the protein concentration was calculated from the calibration curve from different
240 concentrations of bovine serum albumin.

241 The rest of the supernatant (192 μL) was mixed with 148 μL of phosphate buffer and
242 510 μL of a methanol-chloroform solution (ratio 2/1, volume/volume). After
243 centrifugation at 180 g and 4 $^{\circ}\text{C}$ for 10 min, 15 μL of chloroform was transferred to the
244 new microtubes for the triglyceride assays and stored at -20 $^{\circ}\text{C}$. The pools were
245 redissolved into 200 μL of Triton-BSA buffer. The manufacturer's instructions were
246 followed for the triglyceride colorimetric assay (Triglycerides, kit reference CC02200,
247 LTA SRL, Italy).

248 For the measurement of total sugars, 80 μL of the methanol-chloroform solution of
249 each pool were dried for 30 min at room temperature before adding 300 μL of fresh
250 anthrone solution (1.42 $\text{g}\cdot\text{L}^{-1}$ anthrone in 70% acid sulfuric solution). Next, the pools were
251 heated at 90 $^{\circ}\text{C}$ for 15 min, and the absorbance was measured at 625 nm. Different glucose
252 concentrations were used for drawing the calibration curve, and total sugar amounts
253 were thus expressed as glucose equivalents.

254 We then calculated total energy content, in mJ, using the energy of combustion
255 (Gnaiger, 1983; de Coen & Janssen, 1997): 17,500 $\text{mJ}\cdot\text{mg}^{-1}$ glycogen, 39,500 $\text{mJ}\cdot\text{mg}^{-1}$ lipid,
256 and 24,000 $\text{mJ}\cdot\text{mg}^{-1}$ protein. We summed the three energy contents to determine the
257 energy, in mJ, per *D. magna* and per mg of *D. magna* (i.e., taking into account the mass
258 differences between each type of individuals).

259 **Reflectance (Measure 6)**

260 We measured *D. magna* reflectance around the midgut where the parasite-induced
261 alteration in the coloration of the body is observable using a spectrophotometer

262 (USB2000+) between 280 and 850 nm (DH-2000 Deuterium Tungsten Source, 210-
263 1700nm), and the SpectraSuite Cross-Platform Spectroscopy Operating Software. We
264 used 80 naturally exposed *D. magna* (40 presenting no visible sign of infection and 40
265 with visible signs) collected in July 2018 from the La Villette pond and kept in rearing
266 tanks for less than 6 hours. We alternately measured five uninfected and five infected *D.*
267 *magna*, removing the water with a towel for a few seconds before the measurement.

268 **Susceptibility to insect predation (Measures 7 and 8)**

269 *Notonecta* sp. (n = 13) were starved for 24 h before the experiments, and *D. magna*
270 were collected from the La Villette pond in July 2018 and used within 6 hours. We used
271 500 mL jars filled with spring water (Cristaline®, Cristal-Roc source) and performed a
272 first experiment on the timing of capture and handling time (Measure 7&8) and a second
273 experiment on prey choice (Measure 9).

274 For the timing of capture (Measure 7), after 24 h of acclimatization for the *Notonecta*
275 sp., we offered three *D. magna* that were either infected or presenting no sign of infection
276 (hereafter healthy) to the *Notonecta* sp. for 1 h. We recorded the times of capture of alive
277 prey and the release of each prey cadaver. We defined handling time (Measure 8) as the
278 time interval between capture and release, and intercapture time as the time interval
279 between the release of the current prey (or the start of the experiment) and the capture
280 of the next prey. We simultaneously offered healthy *D. magna* to half of the *Notonecta* sp.
281 and infected *D. magna* to the other half. After another 24 h period of acclimatization and
282 starvation, we performed the same experiments with the other prey type *per* predator.

283 To investigate prey choice (Measure 9), we offered 10 healthy and 10 infected *D. magna*
284 to each of the 13 *Notonecta* sp. after a 24 h acclimatization and starvation period. When
285 approximately half of the prey was consumed, we stopped the experiment, counted the
286 surviving *D. magna*, and identified their infection status. To determine the preference of

287 the predator for infected prey, we used the Manly's alpha index (Manly, 1974; Goren &
288 Ben-Ami, 2017).

$$289 \quad (1) \quad \alpha_i = \ln p_i / \sum_{j=1}^m \ln p_j$$

290 where α_i is the Manly's alpha for prey type i (the infected prey here), p_i and p_j are the
291 proportions of prey types i and j , respectively, at the end of the trial, and m is the total
292 number of prey (here 2). If *Notonecta* sp. prefers infected *D. magna*, then α_i tends to 1, a
293 α_i value of 0.5 indicating the absence of preference.

294 **Statistical analyses**

295 Statistical analyses were performed using R (version 3.4.3) with a significance
296 threshold of 5%. Data (Measures 1-4) from the experimental infection (fecundity,
297 survival, size, mobility) were simultaneously analyzed with a Multiple Factor Analysis
298 (MFA), because we performed several measures on the same identified individuals, as
299 well as separately as a complement to compare with the results of naturally-infected
300 individuals (see Appendix B). We used 10 parameters aggregated in four factors: Clutch
301 Size/Clutch Frequency/Maturity (Fecundity), Lifespan (Lifespan), Maximal
302 Speed/Average Speed/Number of Turns/Inactivity (Mobility), and Size (Size). Because
303 total egg production results from a combination of fecundity and lifespan traits, we added
304 it as a supplementary parameter as well as the status of infection.

305 In addition to the MFA, we performed a survival analysis on the results of experimental
306 infections (log-rank test) and compared the death age between healthy juveniles (control
307 *D. magna* dead before the first clutch) and exposed juveniles to assess juvenile mortality
308 (Measure 2). For adult mortality (from first clutch to death), we compared the death age
309 (i.e., the survival) between healthy (control), exposed (no characteristic coloration of
310 infection), and infected *D. magna* (with phenotypic signs of infection) and the adult period
311 (from first clutch to death). To quantify the effects on reproduction (Measure 1), we

312 performed a survival analysis (log-rank test) on age at maturity (date of the first clutch)
313 and compared clutch frequency and mean clutch size (i.e., number of eggs/embryos in the
314 brood chamber) between adult categories using the analysis of variance (ANOVA)
315 followed by **one-sided** pairwise t-tests (with the Holm adjustment method) after log-
316 transformation. Total reproduction (total number of clutches and offspring during
317 lifetime) was analyzed using a generalized linear model (GLM) **with a quasi-poisson error**
318 **term and a logarithmic link function**, while we used **one-sided** Tukey contrast for pairwise
319 analyses.

320 To analyze the fecundity of naturally-infected individuals (Measure 1), we considered
321 the abundances of broodless (no egg or ephippia), egg-carrying, and ephippia-carrying *D.*
322 *magna* with (i.e., infected) or without (i.e., healthy) phenotypic signs of infection. Because
323 infection is visible around Day 10, we considered all infected *D. magna* as adults. However,
324 a large proportion of broodless healthy *D. magna* could be juveniles (Hülsmann & Weiler,
325 2000). Thus, using the Lampert's method (described in Stibor & Lampert, 1993) – adult
326 size is the smallest class size where less than 50% are broodless –, we determined adult
327 size and thus the proportion of adults in each pond. We calculated the number of adults
328 in the broodless group based on this proportion. With this correction, we expected to limit
329 the overestimation of infected brooding *D. magna*. We compared the abundances of the
330 infected and healthy groups with a Fisher's exact test, because several groups showed a
331 low abundance.

332 Analyses of mobility (Measure 3: average speed, maximal speed, proportion of
333 inactivity time, turning number), body size (Measure 4), and biochemical composition
334 (Measure 5) were performed with ANOVA and **two-sided** pairwise t-tests using the Holm
335 adjustment method when the residuals were normally distributed. For the size of the
336 individuals from the natural populations (Measure 4), we used a mixed model with

337 sample dates niched in ponds as random effects; we used a GLM with a Gamma error term
338 and an inverse link function to analyze mobility when the residuals were non-normal;
339 each analysis was coupled with the two-sided Tukey contrast for pairwise analyses.
340 Concerning *D. magna* coloration (Measure 6), we found three peaks in the spectrum that
341 were compared between healthy and infected individuals using Wilcoxon signed-rank
342 tests, because data were not normally distributed.

343 We compared search and handling times (Measure 7) by *Notonecta* between infected
344 and uninfected *D. magna* using paired two-sample one-sided t-tests when the data were
345 normally distributed and one-sided Wilcoxon signed-rank tests when they were not. We
346 calculated the Manly's alpha index (Measure 8) and compared it to the theoretical value
347 of 0.5 indicating no prey choice using a one-sided t-test to detect a significant preference
348 for infected over healthy *D. magna*.

349 We finally estimated a value of prey profitability for *D. magna* from the La Villette pond,
350 in mJ/s, using the ratio between the total energy content (in mJ/*Daphnia*) and the
351 handling time by *Notonecta* sp. for both healthy and infected *D. magna*. Based on the data
352 obtained (Measures 5 and 7), 100 healthy and 100 infected *D. magna* were generated
353 using a bootstrapped method (5,000 iterations), allowing for each individual to calculate
354 a profitability. According to the bootstrap method, the 95% confidence interval of prey
355 profitability is delimited by the 2.5% and 97.5% percentiles of the mean profitability
356 distribution. We also, for each iteration, tested the effect of the infection on the predicted
357 profitabilities using Wilcoxon signed-rank tests. We compared the distribution of these p-
358 values to the distribution of p-values calculated from tests on randomized profitabilities
359 (i.e., as a null model), and to a uniform distribution (Bland, 2013) with a Kolmogorov-
360 Smirnov test.

361

Results

362 Experimental infection (Measures 1, 2, 3, and 4)

363 The three groups of *Daphnia magna*, control, infected, and exposed, are phenotypically

364 different (Fig. 1). We can observe that the ellipses of the 95% interval confidence of the

365 means do not overlap (Fig. 1b). To summarize, Control individuals have either a long

366 lifespan and intermediate mobility or high mobility and intermediate lifespan. Exposed

367 individuals are close to the Control but with a lower mobility and an intermediate lifespan.

368 Infected individuals show lower lifespan and fitness (total egg production), and larger

369 size, with varying mobility. Results are similar for natural populations (Appendix B), with

370 no effect on fecundity, lower mobility and higher body size for infected individuals.

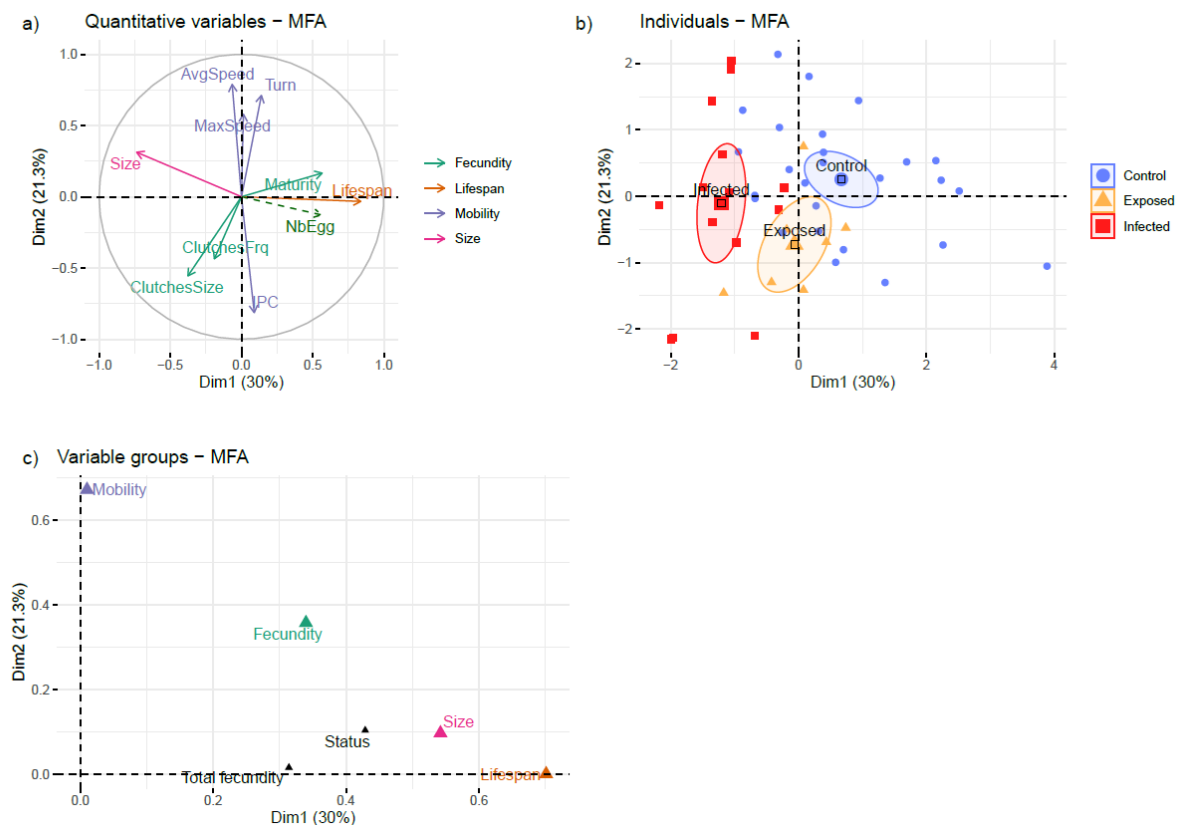


Figure 1. MFA on measurements of *D. magna* experimentally infected with DIV-1 for the two first dimensions. a) Quantitative variables grouped in four categories; note that total egg production (NbEgg) is a supplementary variable. b) Representation of individuals with ellipses for the 95% confidence interval. c) Representation of the group for the two dimensions.

371 In detail, the two first axes of the MFA (30% and 21.3% of the total variation) allow us
 372 to separate the three *D. magna* groups – while the third axis, 16% of the total variation,
 373 does not separate Control and Exposed *D. magna*. The first axis represents (Fig. 1a, 1c)
 374 Lifespan (positively correlated, p-value < 0.001) and Size (negatively correlated, p-value
 375 < 0.001). Note that total egg production is mainly correlated to lifespan, rather than
 376 fecundity parameters. This axis allows separating infected individuals that have a lower
 377 lifespan and a larger size, but a lower egg production, leading to a negative correlation
 378 between lifespan-egg production and size. The second axis corresponds to the *D. magna*
 379 Mobility (negatively correlated, p-values < 0.001 for four parameters). Fecundity can be
 380 described by these two axes: Age at maturity is positively correlated (p-value < 0.001)
 381 and Clutch Size is negatively correlated (p-value = 0.009) to the first axis; Clutch
 382 Frequency (p-value = 0.022) and Clutch Size (p-value < 0.001) are negatively correlated
 383 to the second axis. The first axis is therefore sufficient to separate Infected individuals
 384 from the others, although both the first and second axes are necessary to separate Control
 385 and Exposed individuals.

386 **Biochemical composition and energy value (Measure 5)**

387 We observed similar patterns in the two sampling ponds (p-values (status x pond) >
 388 0.3, Table 2 and Fig. C1). Naturally-infected individuals of *D. magna* had more proteins

Table 2. Host biomass and biochemical composition for the two populations. Means in bold are significantly different at 5% from healthy *D. magna*. See Table C5 for statistical values.

| | | N | Fresh mass | | Proteins | | Lipids | | Carbohydrates | | Total Energy | | | |
|---------------------------|----------|----|-----------------------|--------------|----------------------------|--------------|----------------------------|--------------|----------------------------|--------------|----------------------------|--------------|-----------------------|--------------|
| | | | (mg/ <i>Daphnia</i>) | | (µg/mg of <i>Daphnia</i>) | | (µg/mg of <i>Daphnia</i>) | | (µg/mg of <i>Daphnia</i>) | | (mj/mg of <i>Daphnia</i>) | | (mj/ <i>Daphnia</i>) | |
| | | | mean | (+/- 95% CI) | mean | (+/- 95% CI) | mean | (+/- 95% CI) | mean | (+/- 95% CI) | mean | (+/- 95% CI) | mean | (+/- 95% CI) |
| La Villette, August | Brooding | 8 | 1.62 | (0.12) | 12.14 | (2.90) | 1.68 | (0.58) | 2.23 | (0.23) | 396.83 | (65.26) | 635.86 | (97.19) |
| | Healthy | 8 | 1.48 | (0.16) | 6.88 | (0.83) | 1.17 | (0.32) | 1.10 | (0.15) | 242.69 | (23.56) | 355.05 | (35.22) |
| | Infected | 12 | 1.53 | (0.10) | 15.03 | (2.37) | 1.63 | (0.32) | 1.36 | (0.35) | 449.00 | (53.78) | 675.68 | (60.49) |
| Bercy, May | Brooding | 5 | 1.95 | (0.10) | 12.34 | (1.38) | 1.84 | (0.58) | 0.87 | (0.08) | 383.83 | (40.92) | 751.33 | (105.18) |
| | Healthy | 5 | 1.24 | (0.27) | 9.48 | (1.46) | 1.38 | (0.45) | 0.42 | (0.12) | 289.43 | (51.20) | 354.21 | (99.50) |
| | Infected | 5 | 1.68 | (0.28) | 16.24 | (2.54) | 2.17 | (0.34) | 0.38 | (1.10) | 482.01 | (54.46) | 794.20 | (72.22) |

389 than healthy specimens (p-value < 0.001 for La Villette), but the same amount of proteins
 390 per mg of *D. magna* as healthy brooding *D. magna* (p-value = 0.275 for La Villette).
 391 Infection and brooding did not change the amount of triglycerides, while carbohydrates
 392 are increased in the presence of eggs/embryos alone (p-values < 0.001). To conclude,
 393 brooding and infected *D. magna* had a higher energy content if we consider both energy
 394 per mg of *D. magna* or energy per individual (all p-values < 0.003).

395 Reflectance (Measure 6)

396 The measure of reflectance (Fig. 2), measured in the percentage of reflected light - i.e.,
 397 more the light is reflected, more the individual is colored for each wavelength/color, of
 398 naturally infected *D. magna* clearly shows that the white phenotype is associated with
 399 increased coloration (intensity) both in the UV and visible domains, and to a lesser extent
 400 in the infrared (280 to 850 nm), underlying the higher visibility of infected individuals.

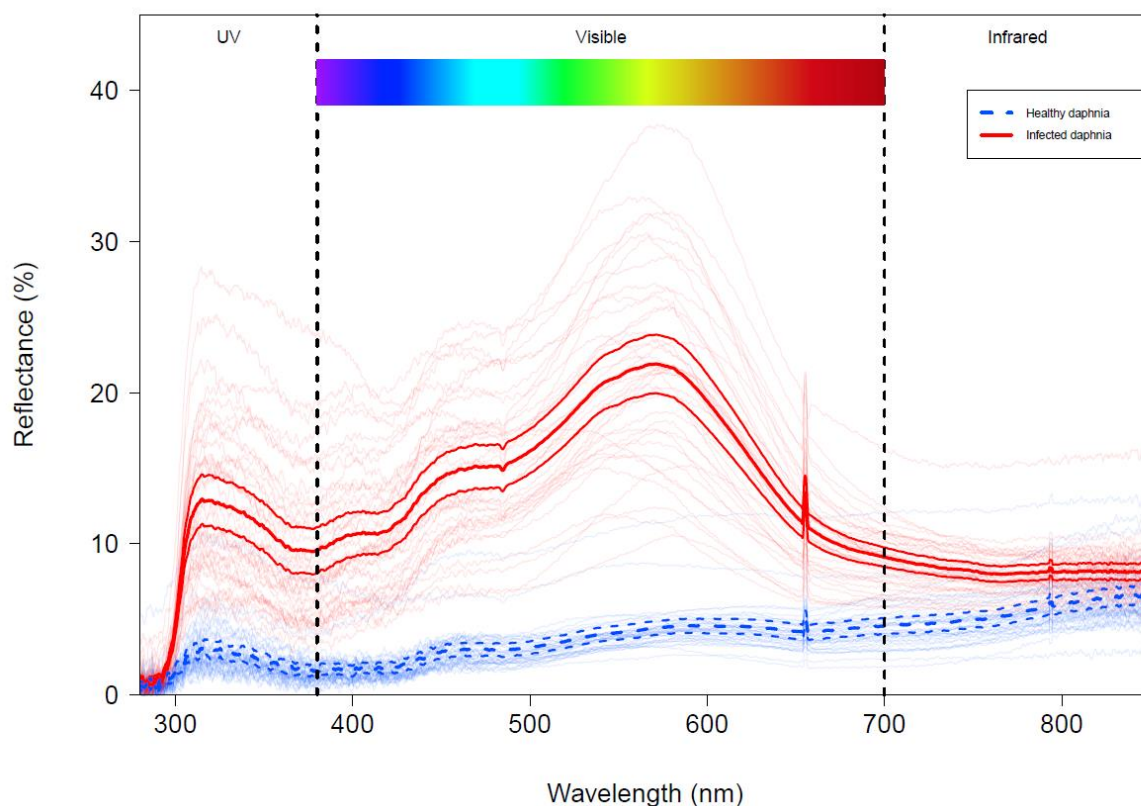


Figure 2. Effects of DIV-1 on reflectance between 280 and 850 nm. Blue (dashed) lines are healthy *D. magna* and red (solid) lines are infected *D. magna*. Highly visible lines are the mean and the lower and upper 95% confidence interval. Weakly visible lines correspond to all the measured *D. magna*. Note the two peaks due to the material (artefacts) around 660 nm and 790 nm. See Table C6 for statistical values.

401 The reflectance of infected *D. magna* was higher (12.19 +/- 4.76%) than that of healthy *D.*
 402 *magna* (3.88 +/- 1.47%). Furthermore, few differences were observed on the position of
 403 the three peaks of reflectance. Three peaks of reflectance were observed for healthy *D.*
 404 *magna*: a first in UV around 317 nm, a second in blue around 460 nm, and a third in orange
 405 around 588 nm. Infection induced a small shift toward green for the blue and orange
 406 peaks (around 477 and 570 nm, respectively; p-values < 0.001) but did not move the UV
 407 peak (around 314 nm, p-value = 0.083).

408 **Vulnerability to predation (Measures 7, 8, and 9)**

409 For both predator species, the time elapsed between two consecutive captures
 410 (Measure 7) did not differ between naturally infected and uninfected *D. magna* (Fig. 3a,
 411 Fig. A1). However, the handling time by *Notonecta* was significantly longer when they

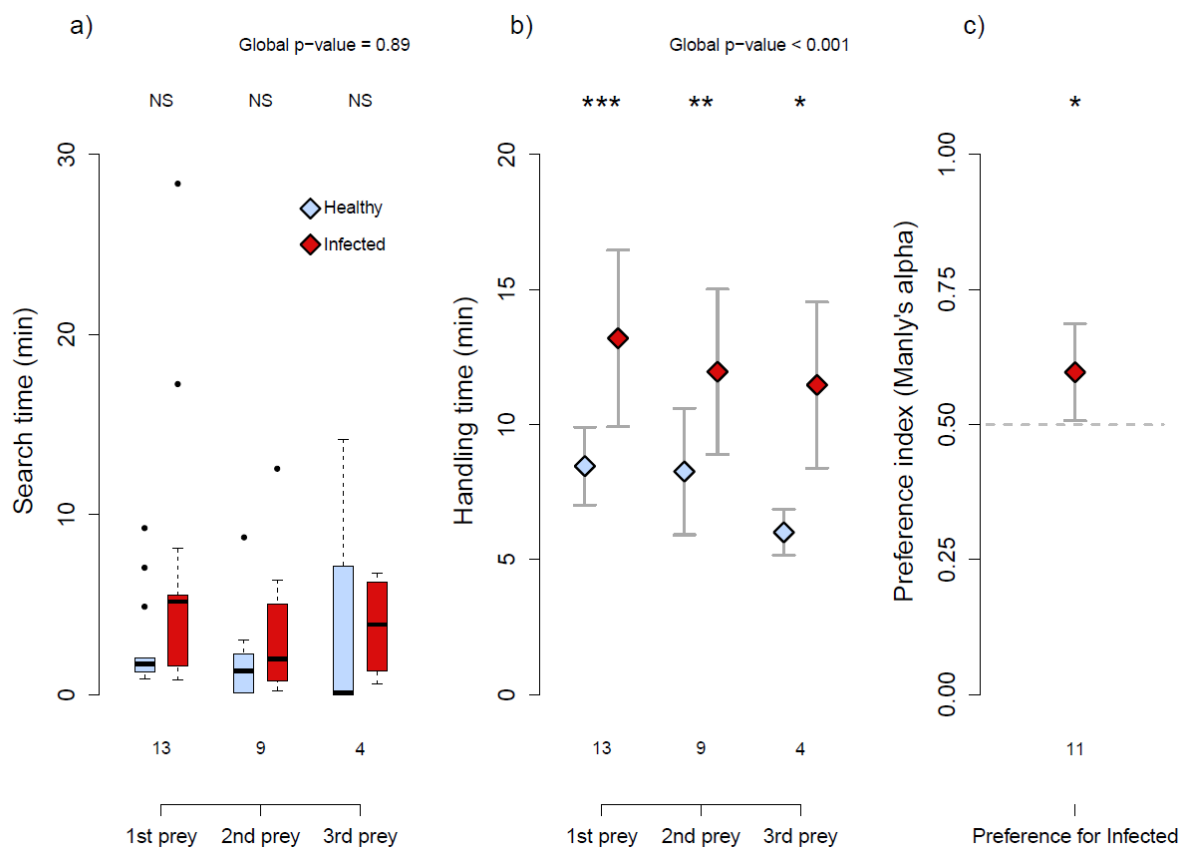


Figure 3. Effects of DIV-1 on vulnerability to predation. a) Search time and b) handling time by *Notonecta* sp., healthy (light blue) or infected (dark red), for the three prey; c) preference for infected *D. magna*. a,b) Statistics compare healthy versus infected prey: dot P < 0.1, *P < 0.05; **P < 0.01; ***P < 0.001; NS P > 0.1. a) Central bars represent the median, boxes the interquartile range, and dots the outliers (> 1.5 times the interquartile range); b,c) dots represent the means and bars the 95% confidence intervals. See Table C7 for statistical values.

412 consumed infected *D. magna* (p-value <0.001 for all catches, Fig. 3b), which are also
413 preferred (Measure 8) over healthy *D. magna* (p-value = 0.03, Fig. 3c).

414 **Prey profitability**

415 Using the values of handling time (Measure 7) and total energy content per *D. magna*
416 (Measure 5), we determined the *D. magna* profitability with a bootstrap analysis. The
417 distribution of p-values of the effect of the infection on bootstrapped *D. magna* is
418 significantly different from the null model and from the uniform distribution (p-values <
419 0.001, Table C8), thus the infection affects the host profitability. Note that the null model
420 is not different from the uniform distribution (p-value = 0.313) as expected (Bland, 2013).
421 Finally, according to the bootstrap, the profitability of healthy *D. magna* is 51.94
422 mJ/*Daphnia* (95% CI: 47.07 – 57.69) and that of infected *D. magna* is 62.86 mJ/*Daphnia*
423 (95% CI: 57.92 – 68.1). Following Cumming & Finch (2005) about the non-superposition
424 of 95% confidence interval, and to the p-values distribution, the profitability of naturally-
425 infected *D. magna* is significantly higher than the profitability of healthy ones.

426 **Discussion**

427 Parasites may affect their host in many ways, with potential repercussions for
428 predators. Here, we investigated the direct and indirect effects of iridovirus DIV-1
429 (*Daphnia* iridescent virus 1) infection in *D. magna* water fleas. We found that DIV-1
430 reduced the survival of water fleas, while the effects on fecundity were not significant. We
431 also noted that infection changed the phenotype of *Daphnia*, mainly by increasing host
432 size, coloration, and energy content. Such changes increased the profitability of infected
433 individuals by 21%. Based on the optimal foraging theory, a preference for infected
434 individuals should be expected, and this assumption is supported by our results. We will
435 after discuss the specific characteristics of “exposed individuals”, those experimentally

436 presented to the virus but displaying no visible sign of infection (white coloration).

437 Finally, we will highlight the complex consequences of parasitism on trophic links.

438 **Reduction of survival but limited effects on vulnerability to predation**

439 The stronger effect of infection concerns the reduction in *D. magna* lifespan. However,
440 there is no obvious effect on fecundity: no change in clutch size or clutch frequency,
441 contrary to previous affirmation of a lower fecundity in the same host-parasite system
442 (Ebert, 2005). The only modification in terms of fecundity characteristics was the earlier
443 age of the individuals at maturity, as previously reported with *D. magna* infected by a
444 microsporidian (Chadwick & Little, 2005). This change could be a plastic modification to
445 compensate for the shorter lifespan (Agnew et al., 1999). Despite this compensation, the
446 total number of offspring was lower for infected *D. magna* compared to control *D. magna*,
447 thus illustrating the negative effect of infection on fitness. In support of our finding, this
448 virulence effect was already observed by Ebert et al. (2000) and Decaestecker et al. (2003)
449 who reported an effect on lifespan and total number of offspring, although these authors
450 did not analyze the effects on clutch size or fecundity. Due to the virus replication and
451 accumulation (Marina et al., 2003; Toenshoff et al., 2018), host physiology and integrity
452 are expected to be largely impaired (Agnew et al., 1999). DIV-1 thus reduced host fitness
453 (i.e., total offspring produced during lifetime) by increasing direct adult mortality, likely
454 contributing to explain its low prevalence in ponds (Decaestecker et al., 2005). No effect
455 on juvenile mortality was observed due to the virus exposure, which supports the
456 previous hypothesis (Agnew et al., 1999; Marina et al., 2003; Toenshoff et al., 2018) that
457 the virus progressively accumulates inside the host and ultimately leads to death.

458 Many phenotypic alterations, such as body size, mobility, and coloration, could lead to
459 indirect effects affecting trophic interactions. Infected individuals are larger; however,
460 this effect is generally observed for infection by castrating parasites (Hall et al., 2007),

461 where the energy not used to reproduce is reallocated to growth. Here, there is no effect
462 on fecundity, meaning that an unknown physiological modification could explain it. A
463 possible explanation would be that lower speeds (higher speeds being generally
464 associated with larger sizes, see Dodson & Ramcharan, 1991) save part of the individual
465 energy budget that can then be reinvested in growth. The difference between ponds may
466 be due to differences in the genotypes of DIV-1 and *D. magna*, as virulence is known to
467 vary with genotypes (Decaestecker et al., 2003). This hypothesis should be tested with
468 experimental infestations for the two populations and also with cross-infestations –
469 combined with genotype analysis. Abiotic conditions may also determine how hosts deal
470 with infection (Bedhomme et al., 2004) and biotic pressure due to predation. We only
471 found *Daphnia* sp. predators (Chaoboridae) in the La Villette pond (pers. obs.) where *D.*
472 *magna* are less active. Because Chaoboridae larvae are ambush predators (Spitze, 1985),
473 fast *D. magna* might encounter more predators and thus be more prone to predation
474 (Gerritsen & Strickler, 1977), leading to the lower speed of this *D. magna* population. As a
475 result, this would mask the differences between healthy and infected individuals. Other
476 works have shown that *Daphnia* sp. speed could affect vulnerability to predation: slow
477 Cladocera are more vulnerable to copepods (Chang & Hanazato, 2003) and fish (O’Keefe
478 et al., 1998). Thus, slower infected individuals would lead to increased predation by
479 *Notonecta* sp. Moreover, due to the structural properties of iridovirus causing iridescence
480 (Williams, 2008), infected *D. magna* showed a higher reflectance in the UV and visible
481 domains than apparently healthy *D. magna*. Infected *D. magna* may thus become more
482 visible (especially considering the larger size of infected individuals) and then more
483 attractive (O’Keefe et al., 1998; Modarressie et al., 2013; Jacquin et al., 2013) for *Notonecta*
484 sp., which has a high visibility in UV (375 nm) and green (520 nm) (Bennett & Ruck, 1970).
485 This is consistent with the observed preference of *Notonecta* sp. for infected *D. magna*. It

486 would be interesting to determine the relative importance of the various phenotypic
487 changes observed in infected individuals. That is, whether predators prefer infected
488 individuals because they are larger, slower, more visible, or due to changes in the
489 energetic contents.

490 **Increase in host energy content leads to higher profitability**

491 Because of the parasite requirements and the host immune response, infection is likely
492 to alter the biochemical composition of the host. For instance, the fungi *Polycaryum laeve*
493 reduces the lipid content of their *Daphnia pulex* hosts (Forshay et al., 2008), while
494 infection by *Polymorphus minutus* (acanthocephalan) increases the triglyceride content of
495 *Gammarus roeseli* (Médoc et al., 2011). The effects of infection seem highly dependent on
496 parasite taxonomy: with the virus infection, we showed that the energy content of
497 infected *D. magna* is higher than that of broodless healthy ones but comparable to that of
498 healthy individuals with eggs. The difference in biochemical composition between
499 infected and uninfected *D. magna* depends on variations in protein content, as infected *D.*
500 *magna* are more nutritious. This could be linked to the virus life cycle that uses the host
501 cellular machinery to produce viral proteins for their capsids with the persistence of the
502 virus in *D. magna* until host death. Otherwise, the immune response of the host using
503 antimicrobial peptides could also result in a higher protein quantity (McTaggart et al.,
504 2009; Rosa & Barracco, 2010; Xie et al., 2016). Although the fat cells of DIV-1-infected *D.*
505 *magna* are described as being larger by Toenshoff et al. (2018), we found no difference in
506 the lipid content between infected and uninfected *D. magna*. Overall, except for the
507 carbohydrates, the biochemical composition of infected *D. magna* was closer to that of
508 brooding *D. magna* compared to uninfected *D. magna*. This effect is magnified by the
509 larger size of infected individuals, leading to the higher energy content of infected *D.*
510 *magna*.

511 Optimal foraging theory predicts that predators should maximize net energy gain
512 (MacArthur & Pianka, 1966; Charnov, 1976a; b). Following our estimations of *D. magna*
513 energy content and handling time by *Notonecta* sp., we approximated *D. magna*
514 profitability to be around 52 and 63 mJ/s for uninfected and infected individuals,
515 respectively, representing an increase of 21%. **Consequently, in spite of a higher handling**
516 **time, possibly due to the fact that the prey are bigger, the large increase in energy content**
517 **leads to a higher profitability for the infected individuals.** Search time, the third parameter
518 of net energy gain is unchanged despite the modifications to host coloration and a possible
519 reduction in mobility (also in the preliminary experiment with fish). Consequently, based
520 on search time, handling time, and energy content, the predator's preference for infected
521 *D. magna* is not surprising. Nevertheless, we also showed that the parasite greatly
522 increased host mortality, probably leading to the low prevalence observed in natural
523 populations (0.5-3%). Thus, high virulence could counterbalance the increase in host
524 profitability, limiting the predation rate on infected prey. In addition, the low prevalence
525 may explain why the meta-analysis of Flick et al. (2016) showed that predators rarely
526 modify their preference for infected prey. Long-term experiments with predators of
527 *Daphnia* while controlling DIV-1 prevalence to dampen parasite direct effects could be
528 undertaken to explore the indirect effects of parasites on predators' diet.

529 **Exposed individuals differ from healthy ones**

530 Some individuals were exposed to DIV-1 but did not exhibit the most visible sign of
531 virus infection: namely, white coloration. Nevertheless, we noted two differences with
532 healthy individuals: a lower lifespan and a lower mobility. We propose three hypotheses
533 to explain these differences. First, they could have been not infected. Results on healthy
534 *D. magna* showed that their lower mobility is positively correlated with a longer lifespan.
535 Therefore, if exposed individuals have escaped infection, because, for instance, they are

536 slower and thus encounter the virus less often, they should have a longer lifespan.
537 However, because exposed *D. magna* have a shorter lifespan, we may suppose that they
538 have been affected by the virus and not only escaped infection. Second, they could have
539 resisted to infection. We observe that this resistance results in a low lifespan reduction
540 (due to the infection, because the virus does not accumulate in the host) but also a greater
541 mobility reduction (again due to the infection). Both effects may occur because resistance
542 (immunity) is energetically costly. Dallas et al. (2016) showed the “cost of resistance”
543 (lifespan reduction) on various *Daphnia* sp. exposed to *Metschnikowia bicuspidata* (fungi).
544 On the contrary, Labbé et al. (2010), with their experiment of *D. magna* infected by the
545 bacteria *Pasteuria ramosa*, did not observe such costs. A third hypothesis is that DIV-1
546 effectively infects specimens of *D. magna* without inducing the white phenotype. Studies
547 on iridovirus named this effect as “covert infection” as opposed to “patent infection”
548 (Williams, 1993; Marina et al., 1999; Williams et al., 2005). We conclude from these
549 observations that there are not two extreme categories (i.e., healthy and infected) with a
550 gradient of intensity of parasitic effects but rather various combinations of effects
551 depending on how the host react to infection. Clarifying this aspect would require testing
552 if exposed individuals are infected or not, using microscopy or PCR techniques (Toenshoff
553 et al., 2018).

554 **On the complexity of adding parasites to predator-prey relationships**

555 In this work, we showed that a non-trophic-transmitted parasite could affect its host
556 in many ways. Adding to the well-known effect of virulence (i.e., higher mortality), we
557 showed morphological, behavioral, and physiological effects. These less studied effects
558 result in an increase in energy profitability. Thus, at larger scales, two effects are expected
559 considering the optimal foraging theory. The increase in profitability should lead to an
560 increase in host predation. On the contrary, if higher mortality leads to a decrease in host

561 availability, then predation on the host should decrease. Higher mortality also results in a
562 reduction in competitive ability (Decaestecker et al., 2015). While the evolutionary
563 investigations of the predator's diet go beyond the scope of the present article, theoretical
564 work suggests that parasite effects could lead to antagonistic modifications in predator
565 diet: the increase in host vulnerability should favor predation on the host contrary to the
566 increase in host mortality (Prosnier et al., 2020). It would be interesting to perform
567 experiments with and without infection dynamics, that is, by fixing or not fixing host
568 density or parasite prevalence to separately consider the effects on host energy and host
569 availability. Such experiments would also offer a way to understand how predation on
570 host affects parasite dynamic, the conditions under which it reduces infection (healthy
571 herd hypothesis, Packer et al., 2003) or when it favors the dispersal of a non trophically-
572 transmitted parasite, as *Chaoborus* do for the spores of a *Daphnia's* fungal parasite
573 (Cáceres et al., 2009).

574 A second interesting point is the existence of a more complex structure in the host
575 population: exposed individuals with cryptic phenotypes that are rarely studied in
576 experimental work (partly due to the difficulty in identifying them) despite their high
577 prevalence compared to individuals with visible signs of infection (Marina et al., 1999;
578 Williams et al., 2005). In theoretical work, there are interesting studies on various
579 epidemiological models (like SEIR), which could be adapted by taking into account the
580 category of exposed individuals. Thus, in the continuity of this study, we question how
581 this third category is important in *D. magna* populations, how they are affected in terms
582 of energy content, and thus what are their consequences in terms of predator diet and at
583 larger scales.

584 Finally, we encourage studies to be conducted at a larger scale, considering that prey
585 infection has repercussions on predators (Flick et al., 2016), thus leading to a modification

586 of trophic links. As shown in many food web studies, it is crucial to understand the
587 implications on community composition, stability, and functioning (McCann, 2000;
588 Kondoh, 2003; Frainer et al., 2018).

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602 **Conflict of interest disclosure**

603 The authors declare they have no conflict of interest relating to the content of this article.
604 Nicolas Loeuille and David Renault are recommenders for PCI Ecology.

605 **Data, script and code availability**

606 Data, script and code are available on Zenodo. DOI: 10.5281/zenodo.7685787 (Prosnier *et al.*
607 2022)

608

Supplementary information

609 Supplementary information is available after the references:

610 - Appendix A: Vulnerability to fish predation

611 - Appendix B: Comparative analysis for experimentally and naturally infected individuals

612 - Appendix C: Supplementary figure and tables of statistics

613

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Appendix A: Vulnerability to fish predation

813

814 We did not observe the effects of infection on the intercapture time of *Notonecta* sp.
815 despite the color modification of *Daphnia magna*. Thus, in line with our hypothesis, we
816 tested whether it could affect the intercapture time of an aquatic vertebrate: the European
817 minnow (*Phoxinus phoxinus*). Using another predator that varies in terms of size, mobility,
818 vision, and hunting method is more representative of the diversity of strategies used by
819 *D. magna* in the field.

820 Fish (2.6-3.4 cm in total length) were purchased online (Armorvif, Brittany, France)
821 and kept in a rearing room under natural light at 19 °C, at a density of 1.7 fish.L⁻¹. The
822 water comprised 75% spring water (Cristaline®, Cristal-Roc source) and 25% osmotic
823 water, which was regularly changed (>30% volume per week) and cleaned daily with a
824 net. The fish were fed with commercial food pellets (Goldfish premium, Tetra®), twice a
825 week.

826 Fish (n = 46) were starved for at least 24 h before the experiments to standardize
827 predation. The experiments were performed in an aquarium (34x19x24cm) filled with 10
828 L of water (75% spring water, Cristaline®, Cristal-Roc source, and 25% osmotic water).
829 To resemble the visual environment of the animals, we covered the edges of the aquarium
830 with green plastic and the bottom with brown paper. The length of the aquarium was
831 divided into two equal parts with a central wall made of green plastic: one part of the
832 aquarium contained the fish and the other part three infected or uninfected *D. magna*
833 without eggs. After an acclimation period lasting for 1 h, we removed the central wall to
834 begin the experiment with the fish being allowed to forage for 1 h. Predation events were
835 recorded with a webcam (Logitech HD Webcam Pro C920) and the software OBS Studio
836 (version 21.1.2). We measured the time of each capture, thus the time between the
837 predation events (first, second, and third capture). Each fish experienced the two different

838 types of prey with 1 h between the two experiments. To avoid time and order effect, half
839 of the fish started with healthy *D. magna* and the others with infected *D. magna*. After 1 h,
840 we performed the same experiments with the other prey type per predator.

841 We compared search time by fish using paired two-sample t-tests, because data were
842 normally distributed. Despite the lower search time for the first prey (Fig. A1, p-value =
843 0.04), we did not observe any effect for the second and third prey (p-values > 0.44). Thus,
844 in addition to predation by *Notoneta*, we did not observe any effect of DIV-1 infection on
845 the search time of the European minnow on account of possible differences in coloration
846 or mobility (in this experiment, individual size (p-value = 0.803) is the same for infected
847 and uninfected *D. magna*, contrary to the insect tests).

848

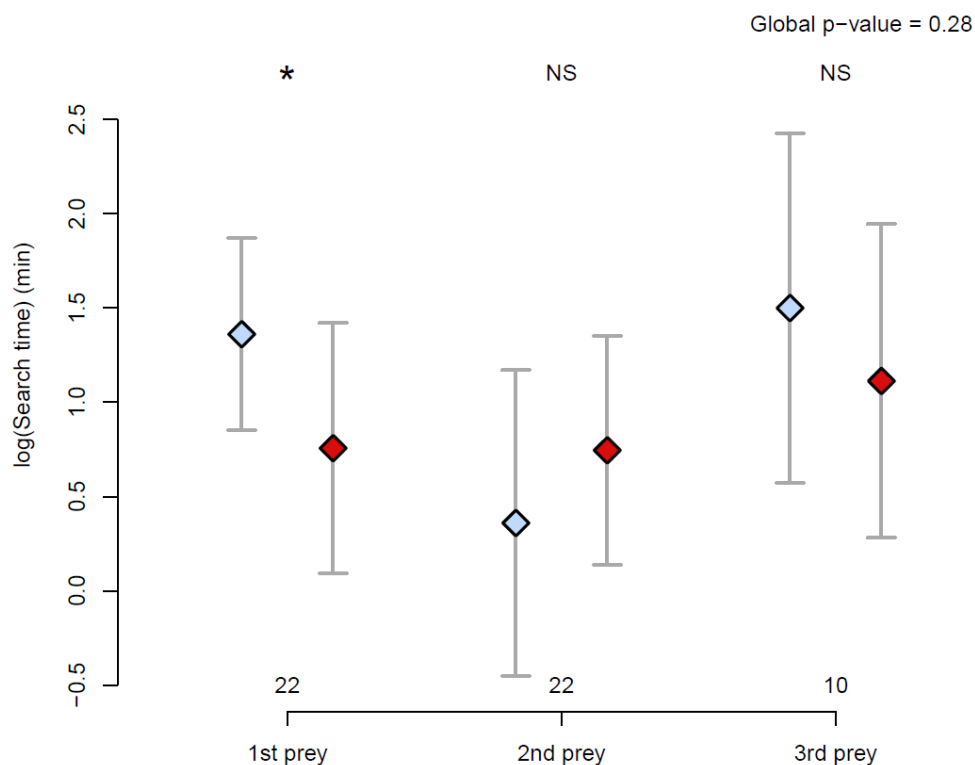


Figure A1. Effects of DIV-1 infection on vulnerability to predation by fish. Search time on healthy (light blue) or infected (dark red) prey for the three prey. Statistics compare healthy versus infected prey: dot $P < 0.1$, * $P < 0.05$; ** $P < 0.01$; NS $P > 0.1$. Dots represent the means and bars the 95% confidence intervals. See Table A2 for statistical values. See Table C7 for statistical values.

849 **Appendix B: Compared analysis of *Daphnia magna* traits for both experimental**
 850 **and natural infection**

851 **Fecundity and mortality (Measures 1 and 2)**

852 Experimental infection (Measure 1) significantly reduced the survival (p-value < 0.001,
 853 Fig. B1a) and adult lifespan (p-value < 0.001) of *D. magna*. DIV-1-exposed individuals (i.e.,
 854 exposed to the parasite but presenting no apparent sign of infection) exhibited
 855 intermediate lifespan and duration of adult life compared to the two other experimental
 856 groups. Exposure to parasites did not affect the mortality of immature *D. magna* (p-value
 857 = 0.319, Fig. B1a). Age at maturity (first clutch) was significantly lower in infected *D.*
 858 *magna* than in controls (p-value = 0.037, Fig. B1b). Exposed individuals were not different

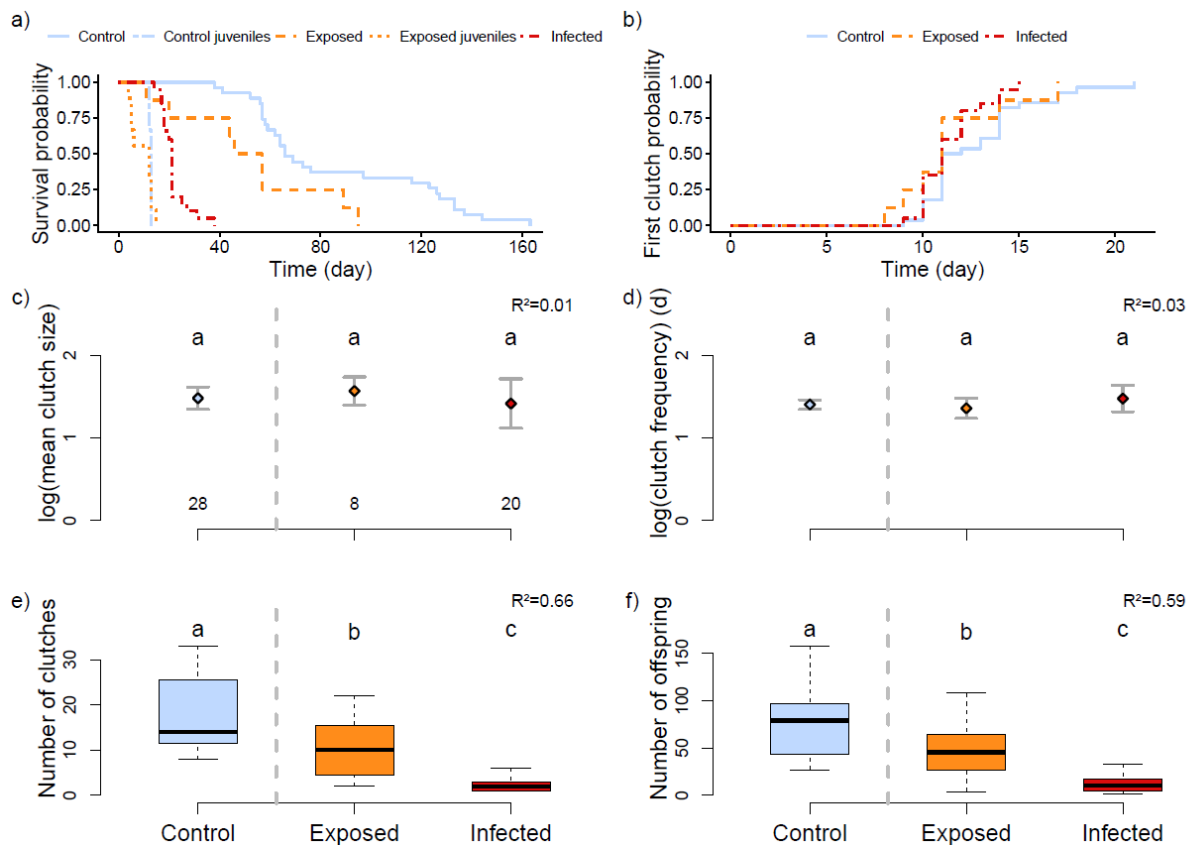


Figure B1. Effects of DIV-1 on host fecundity and survival. a) Survival of *D. magna* depending on infection status (healthy, exposed, or infected) and depending on whether or not they have offspring in their lifetime; b) age at maturity (first clutch); c) clutch size (log); d) clutch frequency (log); e) total number of clutches during lifetime; and f) total number of offspring during lifetime for control, exposed, and infected *D. magna*. The vertical dashed line separates *D. magna* exposed to the control solution (left) and those exposed to the DIV-1 solution (right). Numbers in c) are the numbers of *D. magna* for each category. The same letters indicate the groups that are not significantly different at 0.05. a,b) Representation according to the Kaplan-Meier method; c-d) dots represent the means and bars the 95% confidence intervals; and e-f) central bars represent the median, boxes the interquartile range, and dots the outliers (> 1.5 times the interquartile range). See Table C1 for statistical values.

859 from infected and control individuals in terms of age at maturity. No difference was found
 860 for the mean clutch size (p-value = 0.752, Fig. B1c) and clutch frequency (p-value = 0.489,
 861 Fig. B1d) between each of the groups. DIV-1 significantly reduced the total number of
 862 clutches (p-value = <0.001, Fig. B1e) with an intermediate value for exposed *D. magna*.
 863 Infection reduced total offspring production (p-value < 0.001, Fig. 1f) with an
 864 intermediate value for exposed *D. magna*.

865 For natural populations (Measure 1; Fig B2 and Table C2), after applying the correction
 866 to exclude juveniles using Lampert's method, we did not observe any effect on fecundity
 867 (egg and ephippia production) except for the specimens collected from the Bercy pond on
 868 19 April, which were characterized by higher amounts of ephippia and a lower egg
 869 production for infected *D. magna* (p-value = 0.022), and for those collected from the La

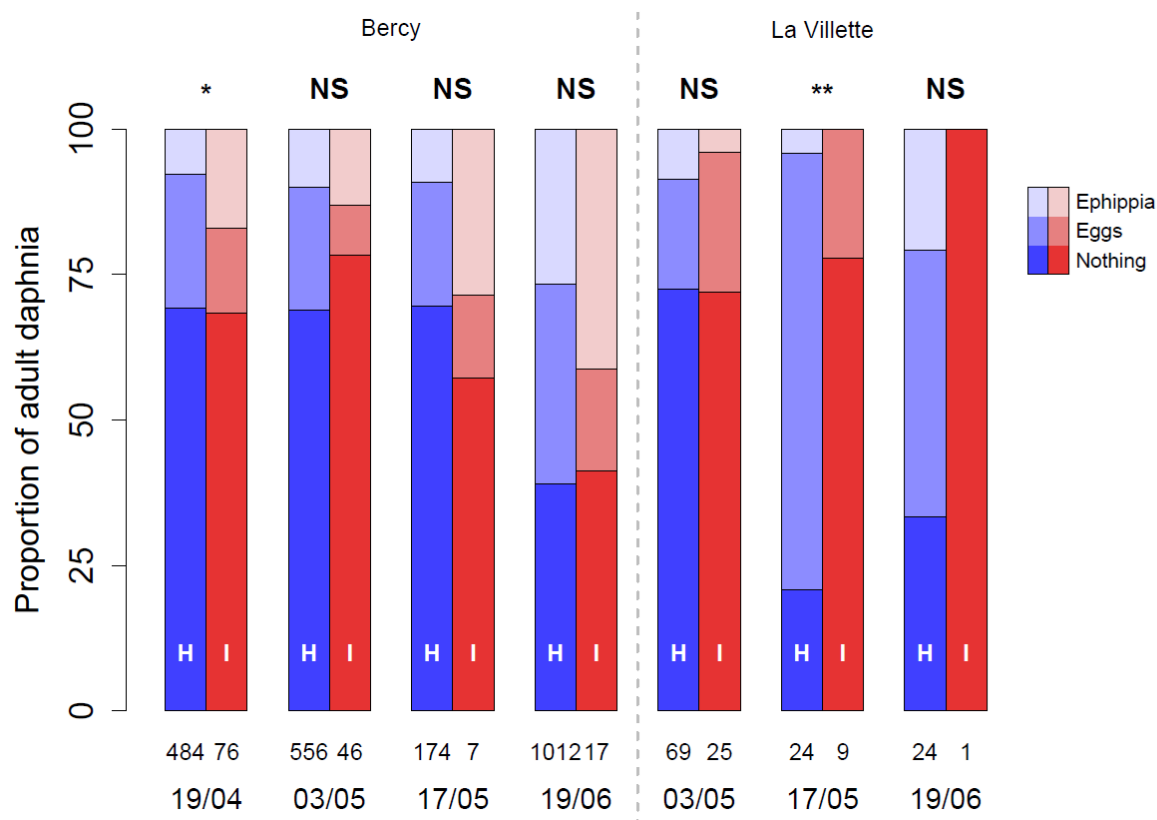


Figure B2. Proportion of adult *D. magna* without eggs, with eggs, or with ephippia depending on their infection status (healthy in blue, infected in red) in the two ponds for various dates. Numbers are the numbers of infected or uninfected *D. magna*. Statistics compare healthy versus infected prey: dot P < 0.1, *P < 0.05; **P < 0.01; NS P > 0.1. See Table C2 for statistical values.

870 Villette pond on 17 May, which had a lower fecundity for infected *D. magna* (p-value =
 871 0.008).

872 **Mobility (Measure 3)**

873 For experimentally infected *D. magna* (Fig. B3a, B3c, B3e), exposed individuals showed
 874 lower activity with a lower mean speed (p-value = 0.008) and a lower maximum speed
 875 (p-value = 0.006), and were more often inactive (p-value = 0.010) than control

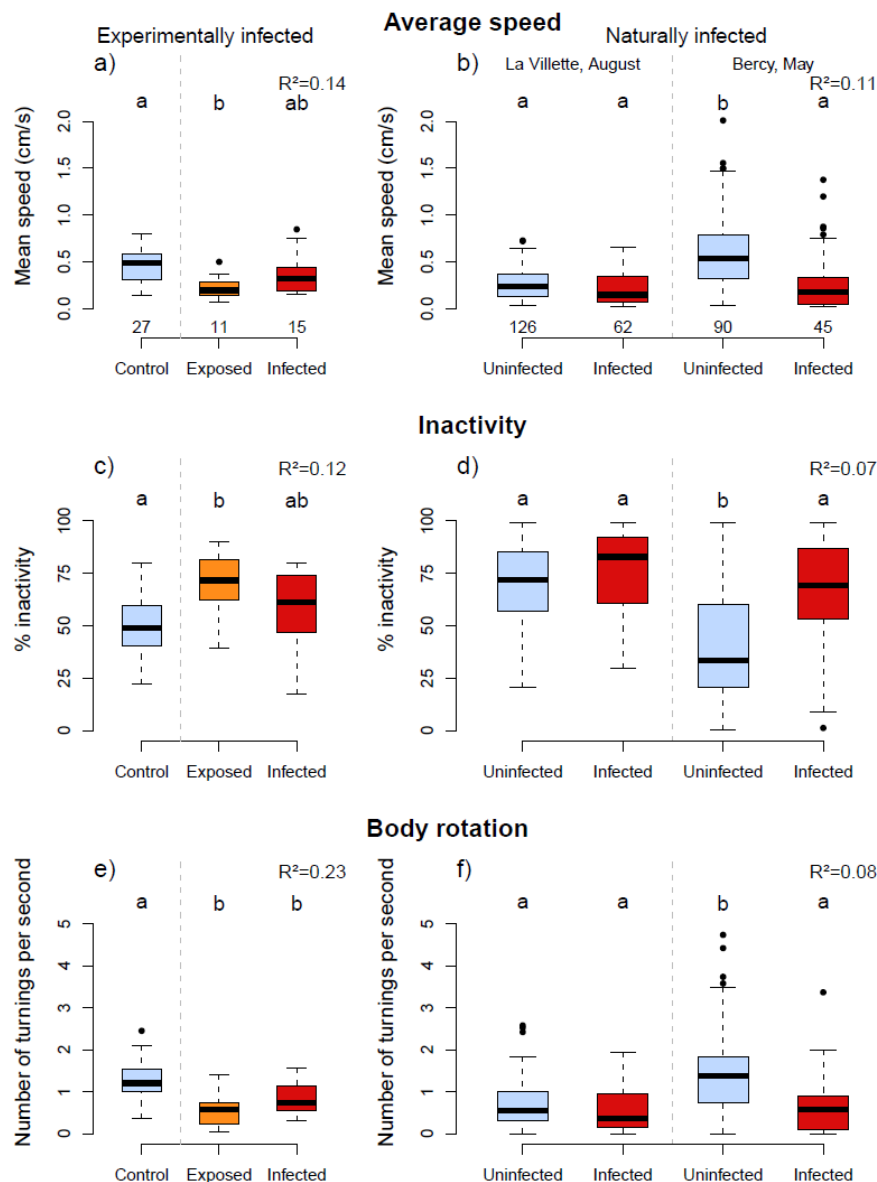


Figure B3. Effects of DIV-1 on host mobility on experimentally infected (left) and naturally infected (right) *D. magna*. a-b) Mean speed; c-d) proportion of inactive time; and e-f) number of turnings for *D. magna* with or without signs of DIV-1 infection. Note that the uninfected category aggregates brooding and unbrooding *D. magna*, because there was no statistical difference in their mobility. Numbers in a-b) are the numbers of *D. magna* for each category. The same letters indicate groups that are not significantly different at 0.05. Central bars represent the median, boxes the interquartile range, and dots the outliers (> 1.5 times the interquartile range). See Table C3 for statistical values.

876 individuals. Conversely, infected *D. magna* showed intermediate activity patterns. The
 877 number of turnings was higher for control *D. magna* compared to infected (p-value =
 878 0.027) and exposed (p-value < 0.001) individuals. For naturally infected *D. magna* (Fig.
 879 B3b, B3d, B3f), there was no significant difference in mobility between uninfected and
 880 infected *D. magna* from the La Villette pond, whereas infected *D. magna* from the Bercy
 881 pond compared to uninfected *D. magna* showed a significant decrease in mean and
 882 maximum speed, activity, and number of turnings (all p-values < 0.001). Note that we
 883 grouped healthy brooding and unbrooding *D. magna* together in the uninfected category,
 884 because eggs/embryos did not modify mobility (all p-values > 0.7).

885 **Body size (Measure 4)**

886 We compared the size of healthy and infected *D. magna* (Fig. B4). For experimentally
 887 infected *D. magna* (same age), infected individuals were larger than controls (Fig. B4a, p-
 888 value = 0.043), while exposed *D. magna* had an intermediate size. For natural populations

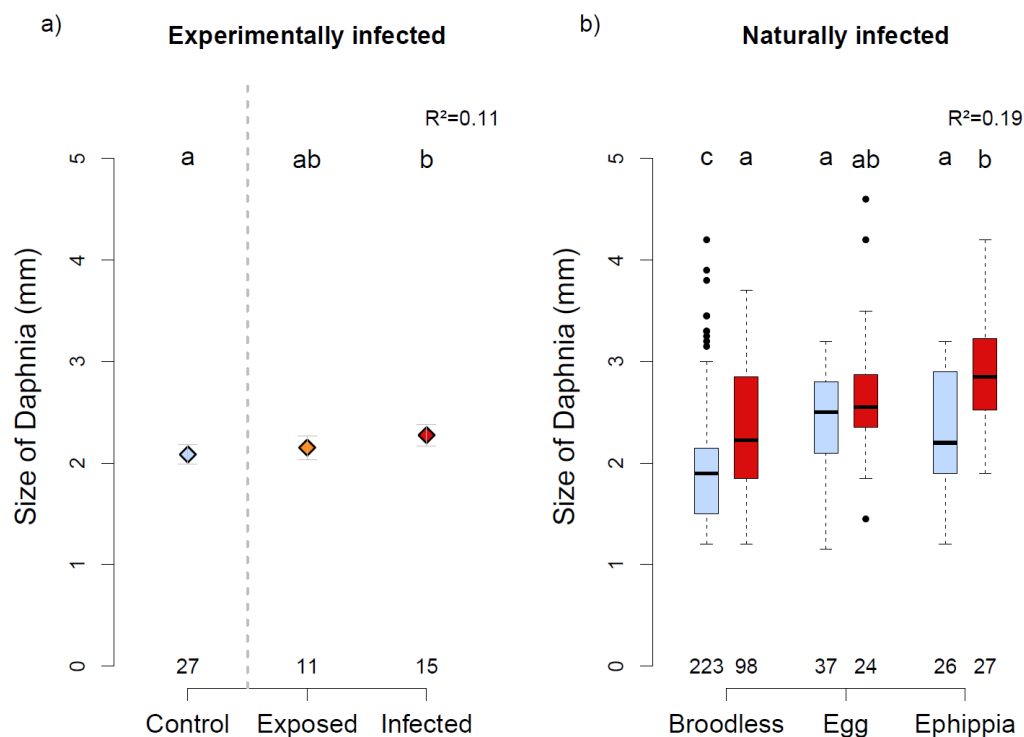


Figure B4. Effects of DIV-1 on host size on a) experimentally infected (healthy/control, exposed, infected); and b) naturally infected *D. magna* (broodless, with eggs, or with ehipippia). Numbers are the numbers of *D. magna* for each category. The same letters indicate groups that are not significantly different at 0.05. a) Central bars represent the median, boxes the interquartile range, and dots the outliers (> 1.5 times the interquartile range); and b) dots represent the means and bars the 95% confidence intervals. See Table C4 for statistical values.

889 (Fig. B4b), we observed the largest sizes with infected individuals that were broodless or
890 with ehippia (p-values < 0.01) but not with infected *D. magna* with eggs (p-value = 0.38).
891 Finally, for the two groups of naturally infected individuals used for the predation
892 experiments, only infected *D. magna* used for *Notonecta* sp. predation were larger than
893 healthy individuals (p-value < 0.001).

894

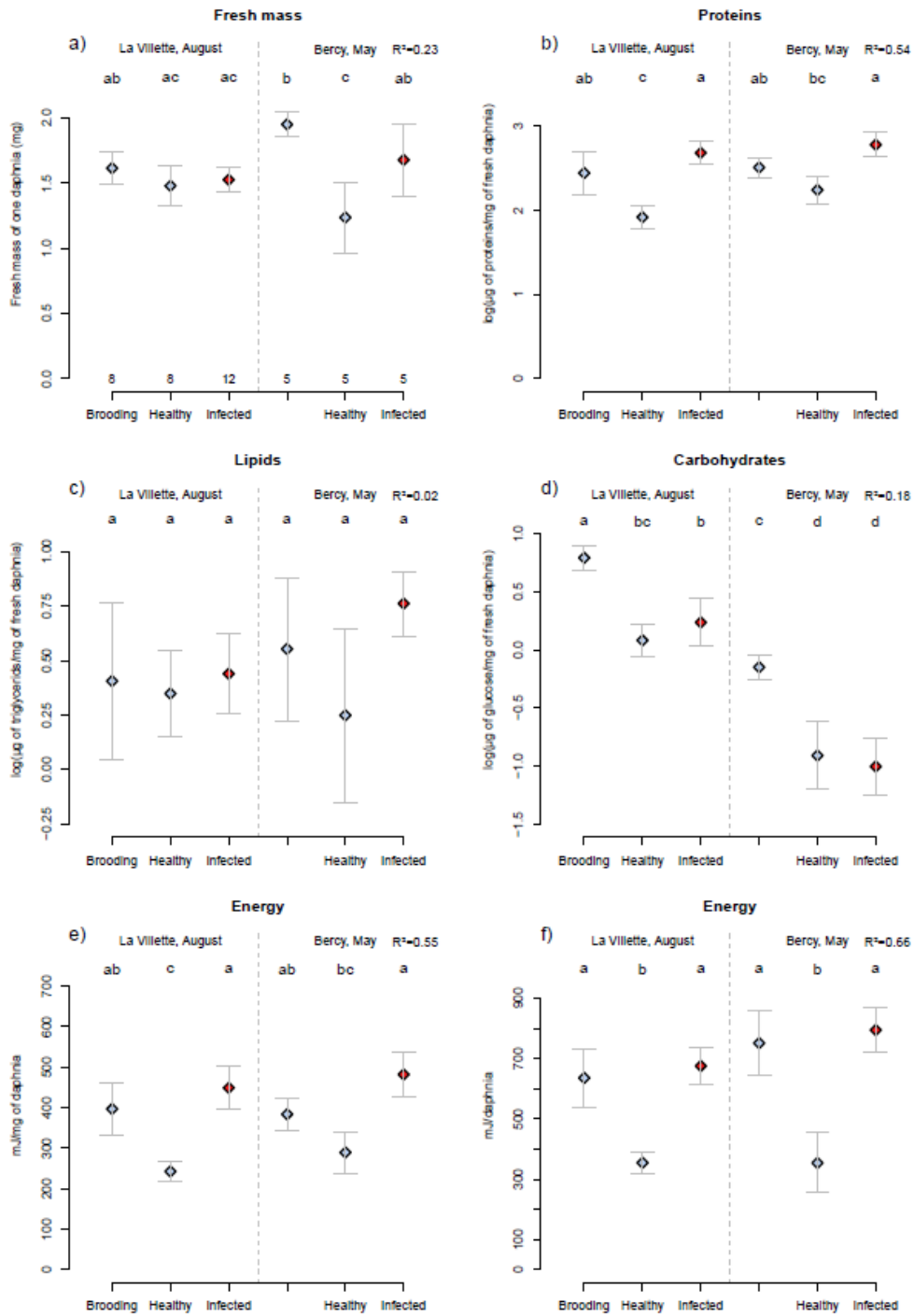


Figure C1. Energy content of *D. magna* for the two populations. a) Biomass, b) protein content, c) lipid content, d) carbohydrate content, e) energy (in mJ) by mg of *D. magna*, and f) energy (in mJ) by *D. magna*. Numbers in a) are the numbers in pools of 10 *D. magna* for each category. The same letters indicate groups that are not significantly different at 0.05. Dots represent the means and bars the 95% confidence intervals. See Table C5 for statistical values.

Table C1. Statistical results of DIV-1 effects on fecundity and mortality for the experimental infection (Fig. B1)

| | | Mortality | | Reproduction | | Fitness | | |
|------------------|----------------|----------------|----------------|-----------------|------------------|------------------|--------------------|---------------------|
| | | Survival | Adult time | Age at maturity | Clutch frequency | Mean clutch size | Number of clutches | Number of offspring |
| | df | 2 | 2 | 2 | 2-51 | 2-51 | 2-52 | 2-51 |
| Global effect | χ^2/F | 58.3 | 61.7 | 4.6 | 0.7247 | 0.2869 | NA | NA |
| | p-value | < 0.001 | < 0.001 | 0.102 | 0.489 | 0.752 | < 0.001 | < 0.001 |
| | R ² | NA | NA | NA | 0.03 | 0.01 | 0.66 | 0.59 |
| Control-Infected | | < 0.001 | < 0.001 | 0.037 | 1 | 0.69 | < 0.001 | < 0.001 |
| Control-Exposed | | 0.01 | 0.011 | 0.252 | 1 | 0.69 | 0.014 | 0.043 |
| Exposed-Infected | | < 0.001 | < 0.001 | 0.78 | 1 | 0.69 | < 0.001 | < 0.001 |

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Table C2. Statistical results of DIV-1 effects on fecundity for naturally infected *D. magna* (Fig. B2)

| Pond | Bercy | | | | La Villette | | |
|---------|--------------|-------|-------|-------|-------------|--------------|-------|
| Date | 19/04 | 03/05 | 17/05 | 19/06 | 03/05 | 17/05 | 19/06 |
| p-value | 0.022 | 0.1 | 0.223 | 0.246 | 0.728 | 0.008 | 0.56 |

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Table C3. Statistical results of DIV-1 effects on host mobility (Fig. B3)

| | | Mean speed | Max speed | Inactivity | Number of turnings |
|-------------------------|-------------------------|----------------|----------------|----------------|--------------------|
| Experimentally infected | | | | | |
| | df | 2-50 | 2-50 | 2-50 | 2-50 |
| Global effect | F | 5.069 | 5.297 | 4.702 | 8.725 |
| | p-value | 0.01 | 0.008 | 0.013 | < 0.001 |
| | R ² | 0.14 | 0.14 | 0.12 | 0.23 |
| Control-Infected | | 0.188 | 0.192 | 0.29 | 0.027 |
| Control-Exposed | | 0.008 | 0.006 | 0.01 | < 0.001 |
| Exposed-Infected | | 0.188 | 0.141 | 0.13 | 0.147 |
| Naturally infected | | | | | |
| | df | 319 | 319 | 3-319 | 319 |
| Global effect | F | NA | NA | 42.32 | NA |
| | p-value (status) | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| | p-value (pond) | < 0.001 | 0.004 | < 0.001 | < 0.001 |
| | p-value (status x pond) | 0.17 | 0.002 | < 0.001 | 0.002 |
| | R ² (status) | 0.11 | 0.07 | 0.07 | 0.08 |
| La Villette, August | Healthy-Infected | 0.18 | 0.07 | 0.22 | 0.566 |
| Bercy, May | Healthy-Infected | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

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Table C4. Statistical results of DIV-1 effects on host size (Fig. B4)

| | | Size |
|-------------------------|-------------------------|------------------|
| Experimentally infected | | |
| Global effect | df | 2-50 |
| | F | 3.223 |
| | p-value | 0.048 |
| | R ² | 0.11 |
| Control-Infected | | 0.043 |
| Control-Exposed | | 0.422 |
| Exposed-Infected | | 0.379 |
| Natural populations | | |
| Global effect | p-value (status) | <0.001 |
| | p-value (egg) | <0.001 |
| | p-value (status x egg) | 0.514 |
| | R ² (status) | 0.19 |
| Healthy-Infected | Broodless | <0.001 |
| | Egg | 0.38 |
| | Ephippia | 0.009 |
| Fish predation | | |
| Global effect | df | 1 |
| | χ^2 | 0.062296 |
| | p-value | 0.803 |
| | R ² | NA |
| Notonecta predation | | |
| Global effect | df | 1-55 |
| | F-value | 25.49 |
| | p-value | <0.001 |
| | R ² | 0.32 |

Table C5. Statistical results of DIV-1 effects on host composition (Fig. C1, Table 2)

| | | Fresh mass | log(Proteins) | log(Lipids) | log(Carbohydrates) | Energy J/mg | Energy J/ <i>Daphnia</i> |
|---------------------|-------------------------|-------------------|-------------------|-------------|--------------------|-------------------|--------------------------|
| | df | 5-37 | 5-37 | 5-37 | 5-37 | 5-37 | 5-37 |
| | F | 6.164 | 12.23 | 1.204 | 40.43 | 10.82 | 20.59 |
| Global effect | p-value (status) | < 0.001 | < 0.001 | 0.242 | < 0.001 | < 0.001 | < 0.001 |
| | p-value (pond) | 0.229 | 0.051 | 0.277 | < 0.001 | 0.3504 | 0.025 |
| | p-value (status x pond) | 0.007 | 0.373 | 0.359 | 0.321 | 0.5862 | 0.28637 |
| | R ² (status) | 0.23 | 0.54 | 0.02 | 0.18 | 0.55 | 0.66 |
| La Villette, August | Healthy-Infected | 1 | < 0.001 | 1 | 0.44 | < 0.001 | < 0.001 |
| | Brooding-Infected | 1 | 0.275 | 1 | < 0.001 | 0.552 | 1 |
| | Healthy-Brooding | 0.965 | 0.002 | 1 | < 0.001 | 0.003 | < 0.001 |
| Bercy, May | Healthy-Infected | 0.032 | 0.015 | 0.54 | 0.583 | 0.003 | < 0.001 |
| | Brooding-Infected | 0.432 | 0.54 | 1 | < 0.001 | 0.361 | 1 |
| | Healthy-Brooding | < 0.001 | 0.54 | 1 | < 0.001 | 0.373 | < 0.001 |

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Table C6. Statistical results of DIV-1 effects on host reflectance (Fig. 2)

| | UV peak | Blue peak | Orange peak |
|---------|---------|-------------------|-------------------|
| df | NA | NA | NA |
| w | 619.5 | 316.5 | 1394 |
| p-value | 0.083 | < 0.001 | < 0.001 |

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Table C7. Statistical results of DIV-1 effects on host vulnerability to predation (Fig. 3 and 910)

| | | Search time | | Handling time | Preference |
|-------------|---------|-------------|-----------|-------------------|-------------|
| | | Fish | Notonecta | | |
| All catches | df | 53 | NA | NA | 10 |
| | t/v | 0.58677 | 127 | 6 | 2.1137 |
| | p-value | 0.28 | 0.891 | < 0.001 | 0.03 |
| 1st catch | df | 21 | NA | 12 | |
| | t/v | 1.8357 | 27 | -4.312 | |
| 2nd catch | p-value | 0.04 | 0.9 | < 0.001 | |
| | df | 21 | NA | -3.2928 | |
| | t/v | -0.77946 | 22 | 8 | |
| 3rd catch | p-value | 0.778 | 0.545 | 0.005 | |
| | df | 9 | NA | 3 | |
| | t/v | 0.58129 | 4 | -3.6364 | |
| | p-value | 0.288 | 0.687 | 0.018 | |

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