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.

Introduction

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

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

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

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

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

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

Pound	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, *Notonecta*)

(Measure 4). We also used naturally-infected individuals to measure fecundity (Measure 1), mobility (Measure

3), size (Measure 4), energy content (Measure 5), coloration (Measure 6), vulnerability to predation (Measure 7&8), and predator preference (Measure 9). Table 1 summarizes the measures performed on each collected *Daphnia*.

Fecundity and mortality (Measures 1 and 2)

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

Gravid *D. magna* collected from the La Villette pond in July 2017 and stored in their rearing tanks were transferred individually to 50 mL jars containing Volvic® water. Newborns (<24h) were transferred individually into jars with 45 mL of Volvic® water in 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, "Erddekokt + 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).

Mobility (Measure 3)

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

Body size (Measure 4)

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

Biochemical composition and energy value (Measure 5)

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

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

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

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

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

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

Reflectance (Measure 6)

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

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

Susceptibility to insect predation (Measures 7 and 8)

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

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

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

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

(1) $$\propto_i = \ln p_i \,/\, \sum_{j=1}^{m} \ln p_j$$

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

Statistical analyses

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

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

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

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

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

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

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

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

Results

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

The three groups of *Daphnia magna*, control, infected, and exposed, are phenotypically different (Fig. 1). We can observe that the ellipses of the 95% interval confidence of the means do not overlap (Fig. 1b). To summarize, Control individuals have either a long lifespan and intermediate mobility or high mobility and intermediate lifespan. Exposed individuals are close to the Control but with a lower mobility and an intermediate lifespan. Infected individuals show lower lifespan and fitness (total egg production), and larger size, with varying mobility. Results are similar for natural populations (Appendix B), with no effect on fecundity, lower mobility and higher body size for infected individuals.

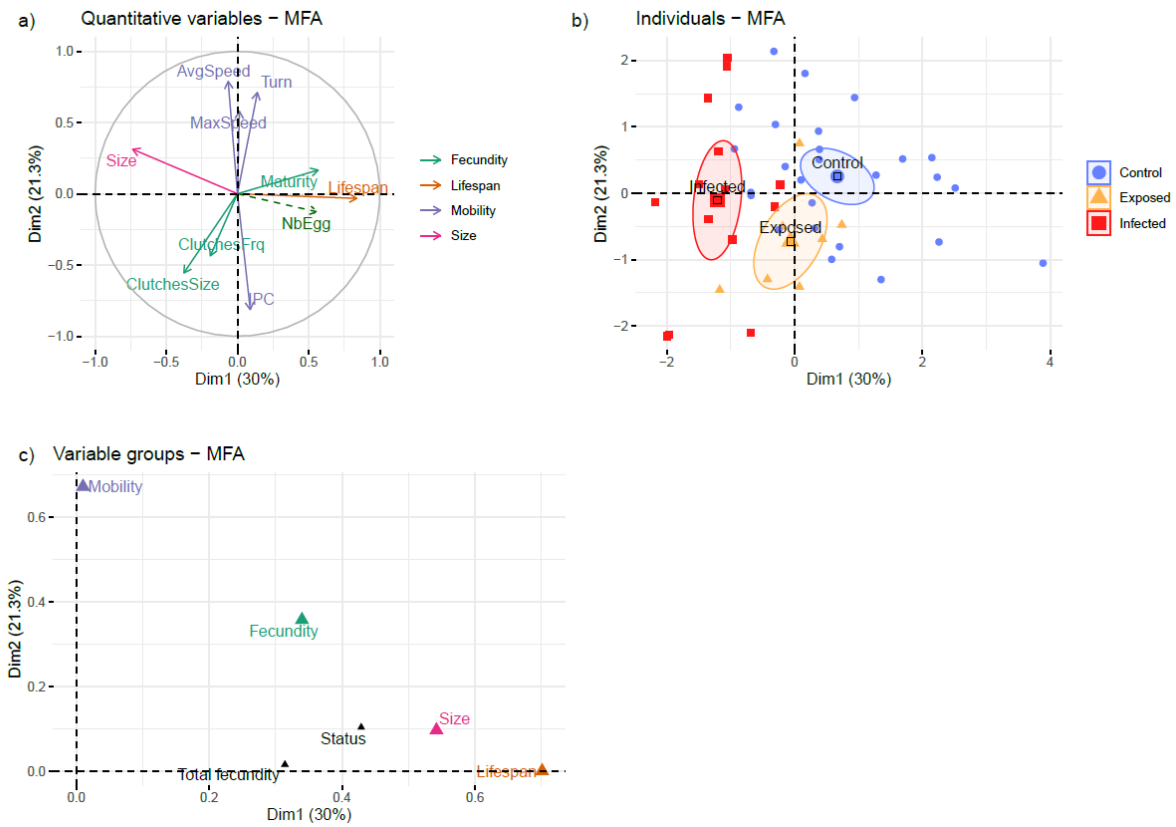

Figure 1. MFA on measurements of *D. magna* experimentally infected with DIV-1 for the two first dimensions. a) Quantitative variables grouped in four categories; note that total egg production (NbEgg) is a supplementary variable. b) Representation of individuals with ellipses for the 95% confidence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

			Fresh mass (mg/*Daphnia*)		Proteins (µg/mg of *Daphnia*)		Lipids (µg/mg of *Daphnia*)		Carbohydrates (µg/mg of *Daphnia*)		Total Energy (mJ/mg of *Daphnia*)		Total Energy (mJ/*Daphnia*)	
		N	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)

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

Reflectance (Measure 6)

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

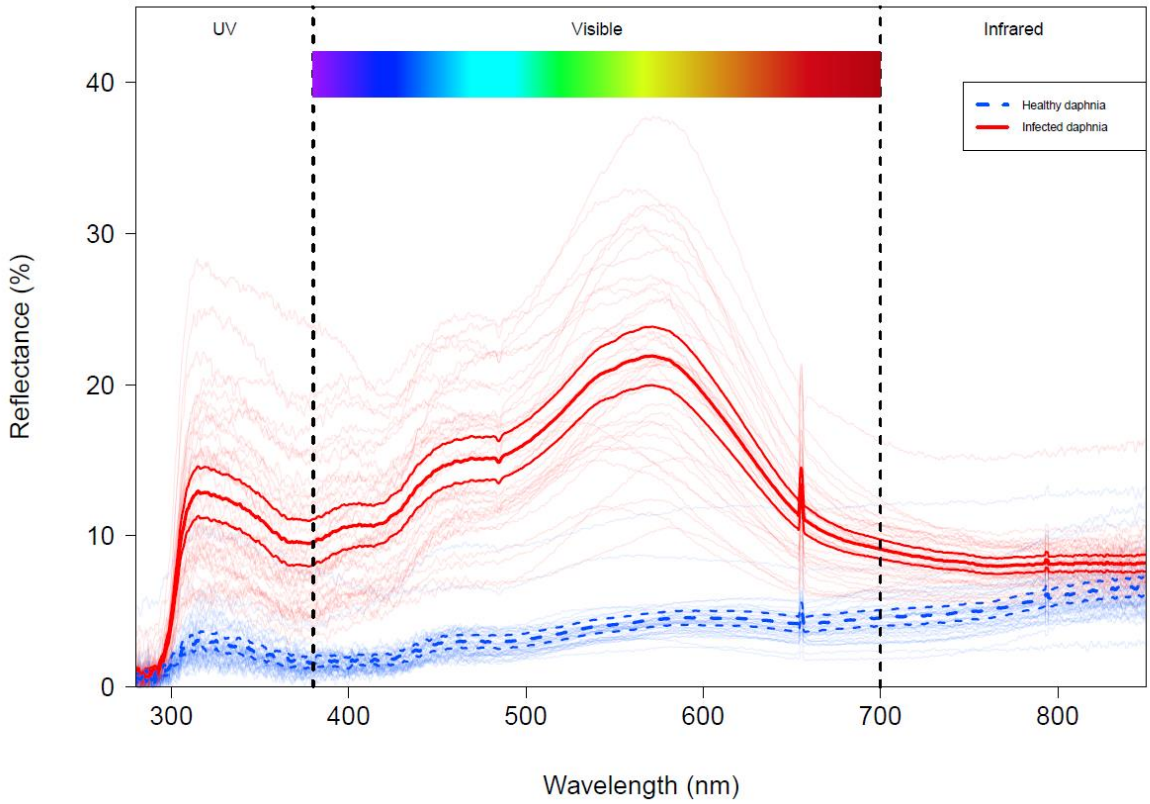

Figure 2. Effects of DIV-1 on reflectance between 280 and 850 nm. Blue (dashed) lines are healthy *D. magna* and red (solid) lines are infected *D. magna*. Highly visible lines are the mean and the lower and upper 95% confidence interval. Weakly visible lines correspond to all the measured *D. magna*.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

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

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

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

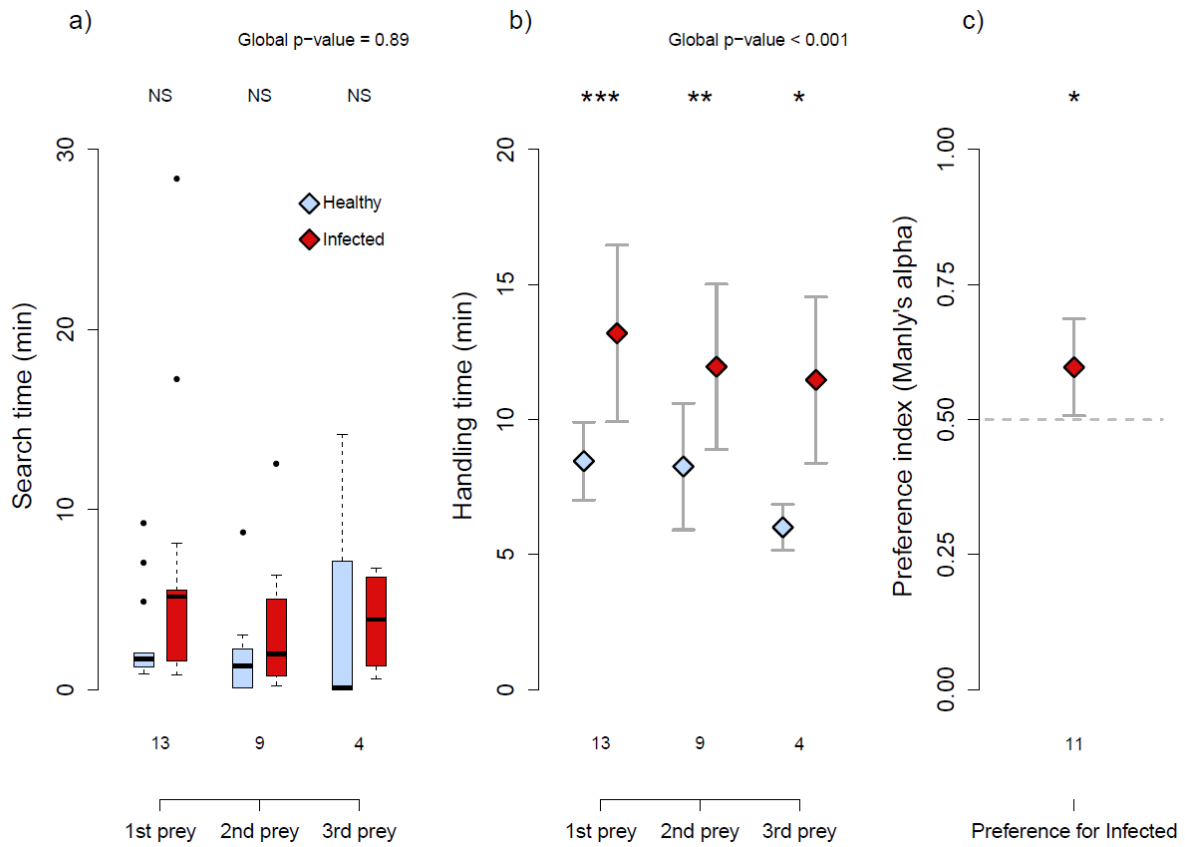

Figure 3. Effects of DIV-1 on vulnerability to predation. a) Search time and b) handling time by *Notonecta* sp., healthy (light blue) or infected (dark red), for the three prey; c) preference for infected *D. magna*. a,b) Statistics compare healthy versus infected prey: dot P < 0.1, *P < 0.05; **P < 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.

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

Prey profitability

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

Discussion

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

presented to the virus but displaying no visible sign of infection (white coloration). Finally, we will highlight the complex consequences of parasitism on trophic links.

Reduction of survival but limited effects on vulnerability to predation

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

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

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

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

Increase in host energy content leads to higher profitability

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

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

Exposed individuals differ from healthy ones

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

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

On the complexity of adding parasites to predator-prey relationships

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

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

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

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

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

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Conflict of interest disclosure

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

Data, script and code availability

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

Supplementary information

Supplementary information is available after the references:

- Appendix A: Vulnerability to fish predation
- Appendix B: Comparative analysis for experimentally and naturally infected individuals
- Appendix C: Supplementary figure and tables of statistics

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Appendix A: Vulnerability to fish predation

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

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

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

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

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

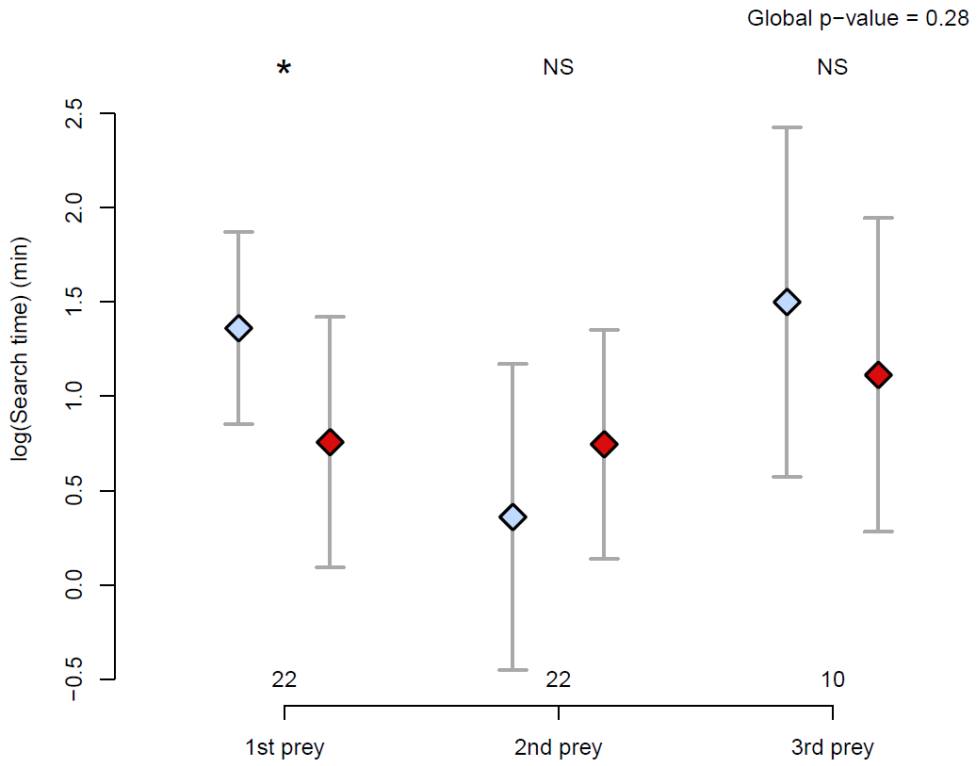

Figure A1. Effects of DIV-1 infection on vulnerability to predation by fish. Search time on healthy (light blue) or infected (dark red) prey for the three prey. Statistics compare healthy versus infected prey: dot P < 0.1, *P < 0.05; **P < 0.01; NS P>0.1. Dots represent the means and bars the 95% confidence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

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

Fecundity and mortality (Measures 1 and 2)

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

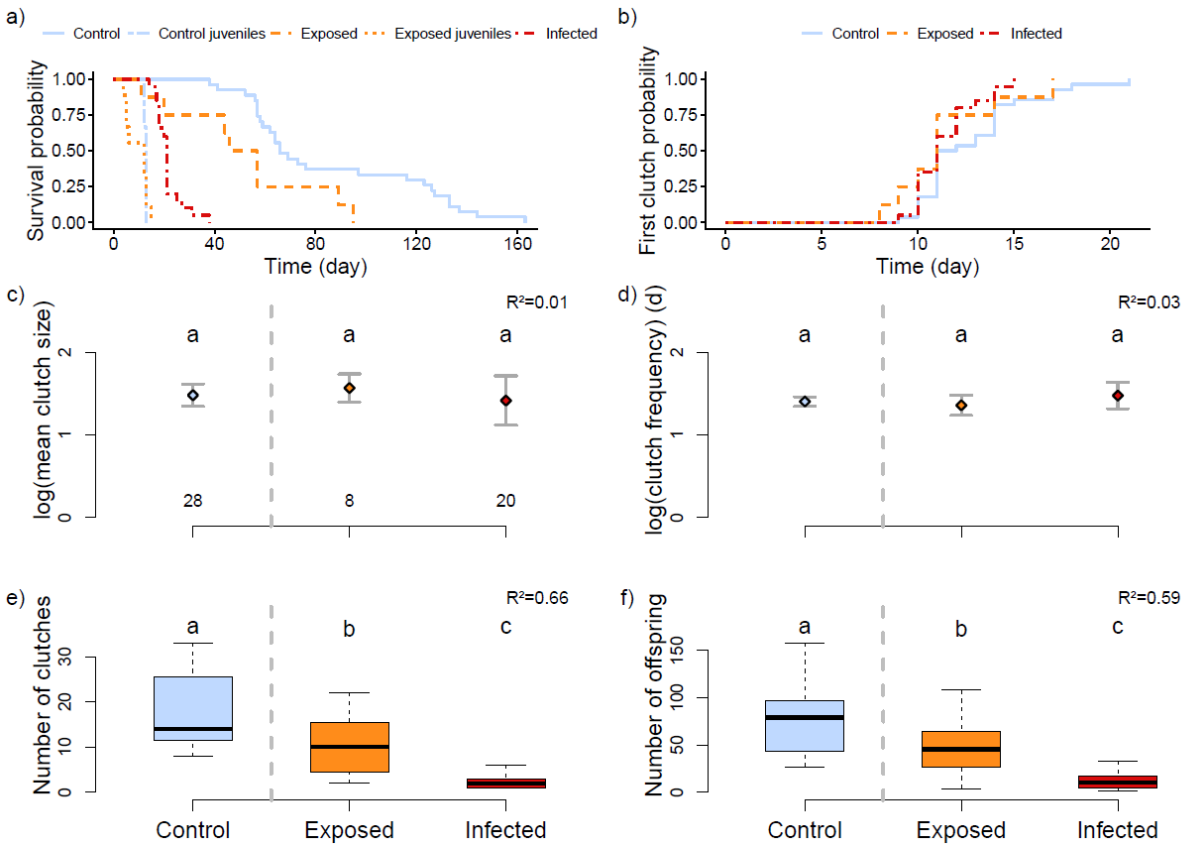

Figure B1. Effects of DIV-1 on host fecundity and survival. a) Survival of *D. magna* depending on infection status (healthy, exposed, or infected) and depending on whether or not they have offspring in their lifetime; b) age at maturity (first clutch); c) clutch size (log); d) clutch frequency (log); 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.

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

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

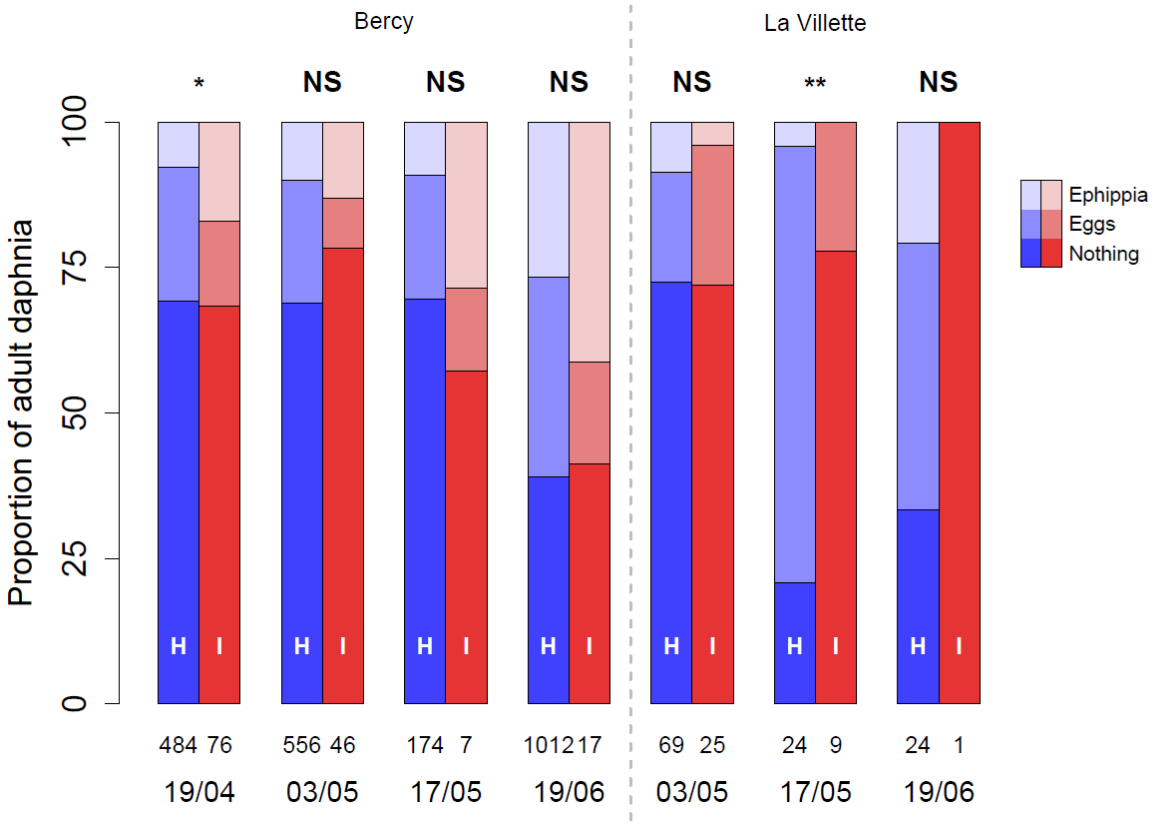

Figure B2. Proportion of adult *D. magna* without eggs, with eggs, or with ephippia depending on their infection status (healthy in blue, infected in red) in the two ponds for various dates. Numbers are the numbers of infected or uninfected *D. magna*. Statistics compare healthy versus infected prey: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

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

Mobility (Measure 3)

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

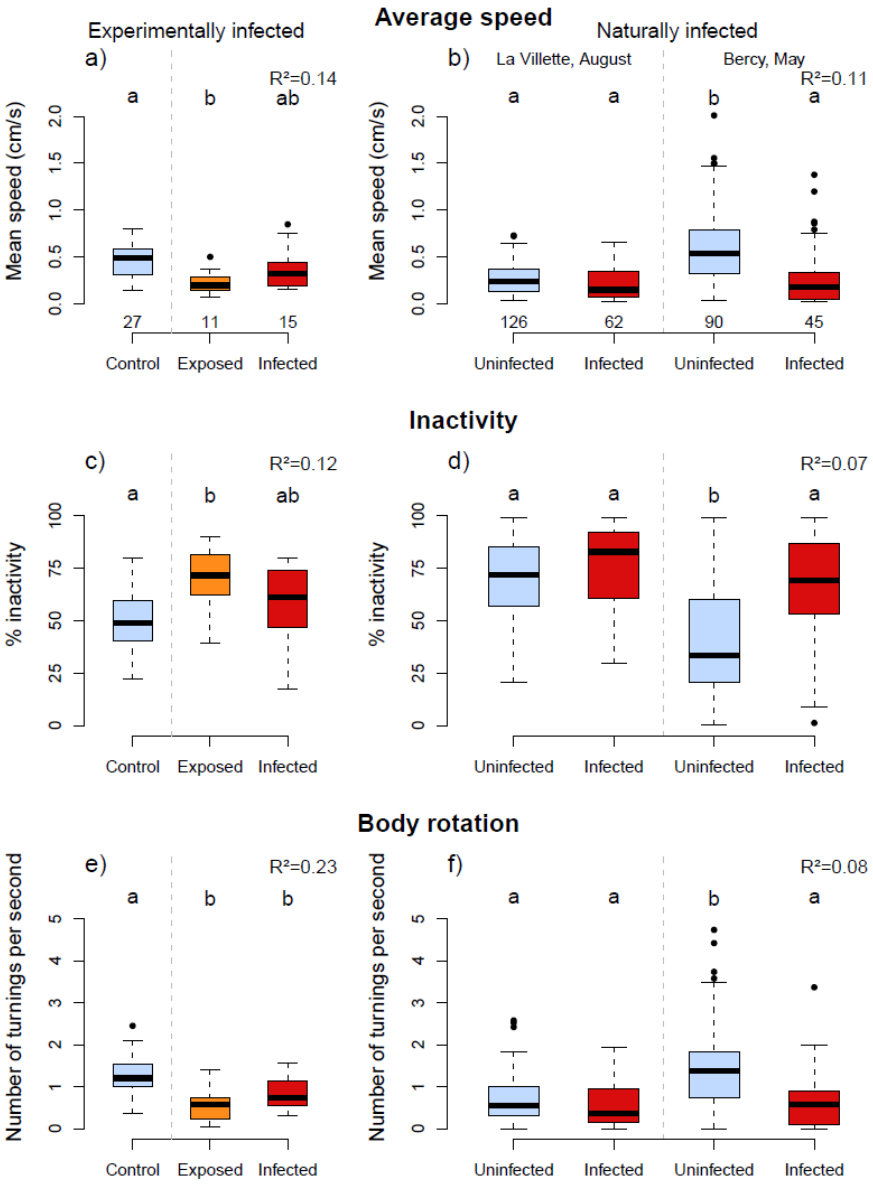

Figure B3. Effects of DIV-1 on host mobility on experimentally infected (left) and naturally infected (right) *D. magna*. a-b) Mean speed; c-d) proportion of inactive time; and e-f) number of turnings for *D. magna* with or without signs of DIV-1 infection. Note that the uninfected category aggregates 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.

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

Body size (Measure 4)

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

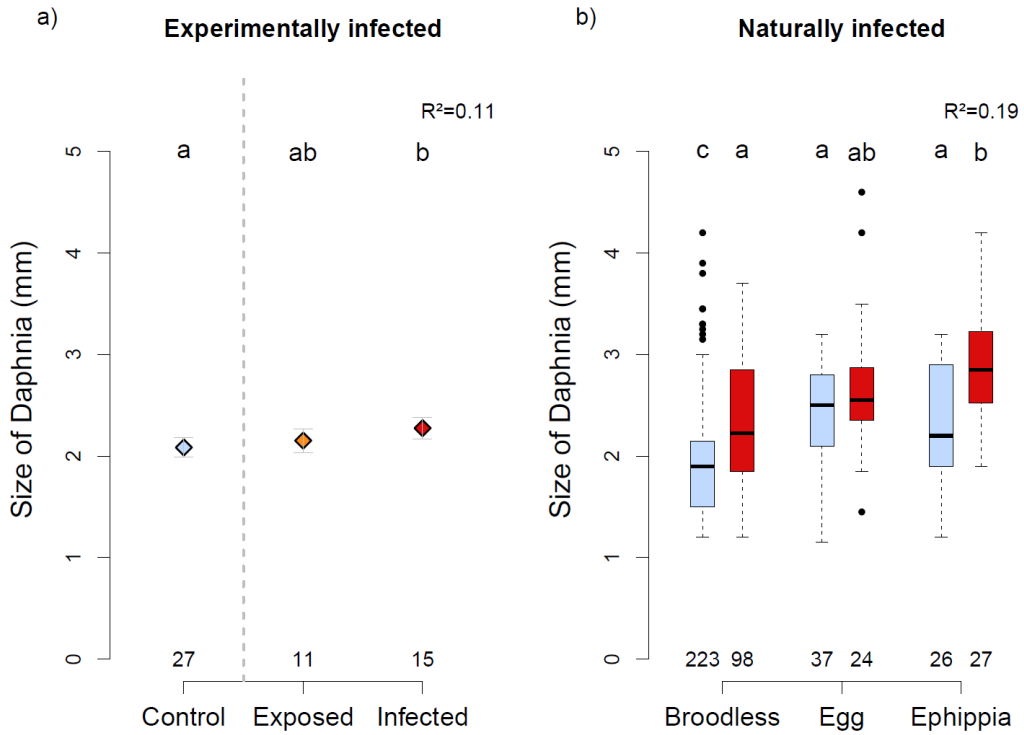

Figure B4. Effects of DIV-1 on host size on a) experimentally infected (healthy/control, exposed, infected); and b) naturally infected *D. magna* (broodless, with eggs, or with ephippia). Numbers are the numbers of *D. magna* for each category. The same letters indicate groups that are not significantly 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.

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

Appendix C: Supplementary figures and tables of statistics

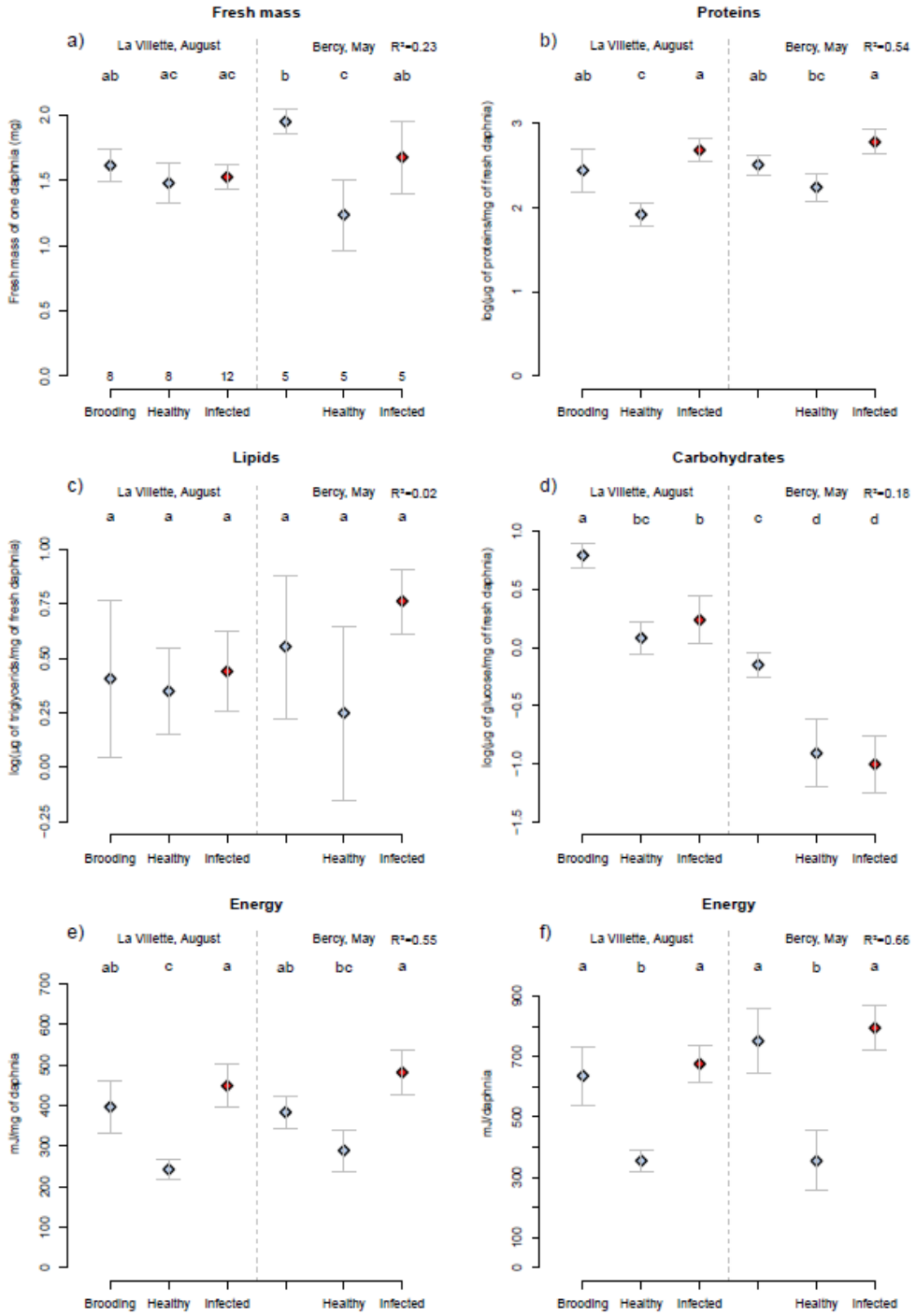

Figure C1. Energy content of *D. magna* for the two populations. a) Biomass, b) protein content, c) lipid content, d) carbohydrate content, e) energy (in mJ) by mg of *D. magna*, and f) energy (in mJ) by *D. magna*. Numbers in a) are the numbers in pools of 10 *D. magna* for each category. The same letters 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
Global effect	df	2	2	2	2-51	2-51	2-52	2-51
	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**

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

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

		Mean speed	Max speed	Inactivity	Number of turnings
Experimentally infected					
Global effect	df	2-50	2-50	2-50	2-50
	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					
Global effect	df	319	319	3-319	319
	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**

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/*Daphnia*
Global effect	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
	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**

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**

Table C7. Statistical results of DIV-1 effects on host vulnerability to predation (Fig. 3 and 9)

		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	
	p-value	**0.04**	0.9	**<0.001**	
2nd catch	df	21	NA	-3.2928	
	t/v	-0.77946	22	8	
	p-value	0.778	0.545	**0.005**	
3rd catch	df	9	NA	3	
	t/v	0.58129	4	-3.6364	
	p-value	0.288	0.687	**0.018**	