# 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 maximizing profitability within the optimal foraging framework. Consequently, parasites could impact predator diet and trophic links within 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 tended to be preferred over uninfected individuals by aquatic insects. Taken together, our findings show that DIV-1 infection could make *Daphnia* more profitable to predators (23% 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.

# 1. 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 (Cezilly et al. 2013). Of particular note, predator 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 and Weyerhaeuser 1987, Newey and Thirgood 2004). For instance, some parasites of water fleas (e.g., fungus, bacteria, trematode) reduce egg production and increase mortality (Schwartz and 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 and 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 and 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 and 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 in a predator's diet depends on its relative abundance and profitability ranking (Emlen 1966, MacArthur and 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 the alteration of the host's behavior and aspect makes them weaker (reducing prey escape) and more visible, 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 the 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 and Sommer 2007, Reynolds 2011). *D. magna* is the host for a diversity of parasites (Green 1974, Ebert 2005), 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 for 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 labelled 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* 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 is further facilitated by the white coloration of the infected water flea individuals.

## 2. Material and Methods

### *2.1. 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%. 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 tanks (100-150 ind.$L^{-1}$) (rearing tanks) 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 start of the experiments. In the main body of the article, we only present the results from the experiments conducted with *Notonecta* sp., while the results from the experiment performed with the European minnow are presented in Appendix A (only one measure of predation was used compared to three measures with *Notonecta* sp.).

In this work, we performed an experimental infection to determine the direct effects of DIV-1 (Measure 1); these data were completed with observational data on naturally infected *D. magna* (Measure 2). We used other naturally infected individuals to measure energy content (Measure 5) and reflectance (Measure 6). We then performed a second set of experiments on

predation (Measures 7 and 8) with naturally infected *D. magna*. We also measured the mobility (Measure 3) of individuals used for Measures 1 and 5, and the size (Measure 4) of individuals used for Measures 1, 2, 7, and 8.

*2.2. Fecundity and mortality (Measures 1 and 2)*

Reproductive success and survival were assessed in two manners: in the laboratory through experimental infections (Measure 1) and from wild individuals (Measure 2).

Measure 1: 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* ($2.3x10^6$ cells.$mL^{-1}$) every 3 days throughout the experiment. These algae were obtained from the Muséum National d'Histoire Naturelle (Paris, France, algothèque MNHN; strain number: ALCP n°349), and cultivated at 20 °C under a 12:12 light:dark cycle in an ES medium (Basal Medium, "Erddekokt + Salze" described by Culture Collection of Algae of Sammlung von Algenkulturen Göttingen). Molts were removed daily to maintain water clarity.

To infect *D. magna*, we prepared a solution of infected *D. magna* cadavers (hereafter, parasite solution) homogenized at the concentration of 1 cadaver/mL in Volvic® water. A control solution was prepared with healthy cadavers. Half of newborns were exposed to the parasite solution and the other to the control solution. On day 1, we added 1 mL of the solution to obtain a ratio of 1 cadaver/juvenile of *D. magna*. On days 4 to 6, we stirred the water (both the control and treatment) using a pipette to resuspend the spores and promote infection. Water was replaced on day 15 by clean water (without the virus) and then once a week until the death of the last individual of *D. magna* (163 days). Offspring were removed and counted daily, and dead *D. magna* were controlled visually, as described above, for infection signs. We started two

sets of experimental infections with 1 day of delay: the first set was performed with 27 juveniles (14 exposed to the parasite solution and 13 to the control solution) coming from 11 distinct mothers, while the second set was performed with 44 juveniles (23 exposed to the parasite solution and 21 to the control solution), also coming from 11 distinct mothers.

Measure 2: Collection took place in April-June 2018 in the two ponds (Bercy and La Villette). We sampled 20 L of water filtered with a 50 µm net to collect *D. magna*. After separating infected and non-infected *D. magna*, individuals were fixed using glycerol solution (1% glycerol, 70% ethanol, 29% water). We then categorized individuals as broodless (without eggs or ephippia), egg-carrying (with parthenogenetic eggs), and ephippia-carrying (with sexual ephippia).

### *2.3. 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 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).

### *2.4. Body size (Measure 4)*

To measure the individual size (from the head to the start of the caudal spine) of the experimentally infected *D. magna* used for Measure 1, 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 2, n = 435). We also used the photographs of a set of *D. magna* used in 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).

### *2.5. Biochemical composition and energy value (Measure 5)*

We assessed the quantity of carbohydrates, lipids, and proteins 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). 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 and 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*.

*2.6. 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 any water with a towel for a few seconds before the measurement.

*2.7. 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) and a second experiment on prey choice (Measure 8).

Measure 7: For the timing of capture, after 24 h of acclimation for the *Notonecta* sp., we proposed 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 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 proposed healthy *D. magna* to half of the *Notonecta* sp. And infected *D. magna* to the other half. After another 24 h period of acclimation and starvation, we performed the same experiments with the other prey type per predator.

Measure 8: To investigate prey choice, we offered 10 healthy and 10 infected *D. magna* to each of the 13 *Notonecta* sp. After a 24 h period of acclimation and starvation. 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 and Ben-Ami 2017).

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

where $\propto_i$ is the Manly's alpha for prey type *i*, $p_i$ 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). In the following, prey I is the infected prey. Thus, if *Notonecta* sp. prefers infected *D. magna*, $\propto_i$ tends to 1. A $\propto_i$ value of 0.5 indicates no preference.

### *2.8. Statistical analyses*

Statistical analyses were performed using R (version 3.4.3) with a significance threshold of 5%. Data from the experimental infection (fecundity, survival, size, mobility) were simultaneously analyzed with Multiple Factor Analysis (MFA, Appendix B) as well as separately as described below. We performed survival analysis on the experimental infection results (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 1). 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, we performed 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 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 log function as the link function for quasi-Poisson distribution, while we used Tukey contrast for pairwise analyses.

To analyze the fecundity of naturally infected individuals (Measure 2), 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 and Weiler 2000). Thus, using Lampert's method (described in Stibor and 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 thus 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 abundance of infected and healthy groups with 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 pairwise t-tests using the Holm adjustment method when residuals were normally distributed. For the size of individuals from the natural populations

(Measure 2), we used a mixed model with ponds and sample dates as random effects; we used a GLM with an inverse function as the link function for a Gamma distribution to analyze mobility when residuals were non-normal; each analyze was coupled with the 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 t-tests if data were normally distributed and Wilcoxon signed-rank tests if 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 t-test to detect the significant preference for infected or 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 mean total energy content (in mJ/*Daphnia*) and the mean handling time by *Notonecta* sp. for both healthy and infected *D. magna*.

# 3. Results

### *3.1. Fecundity and mortality (Measures 1 and 2)*

Experimental infection (Measure 1) significantly reduced the survival (p-value < 0.001, Fig. 1a, Appendix B) 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. 1a). Age at maturity (first clutch) was significantly lower in infected *D. magna* than in controls (p-value = 0.037, Fig. 1b). Exposed individuals were no different 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. 1c) and clutch frequency (p-value = 0.489, Fig. 1d) between each of the groups. DIV-1

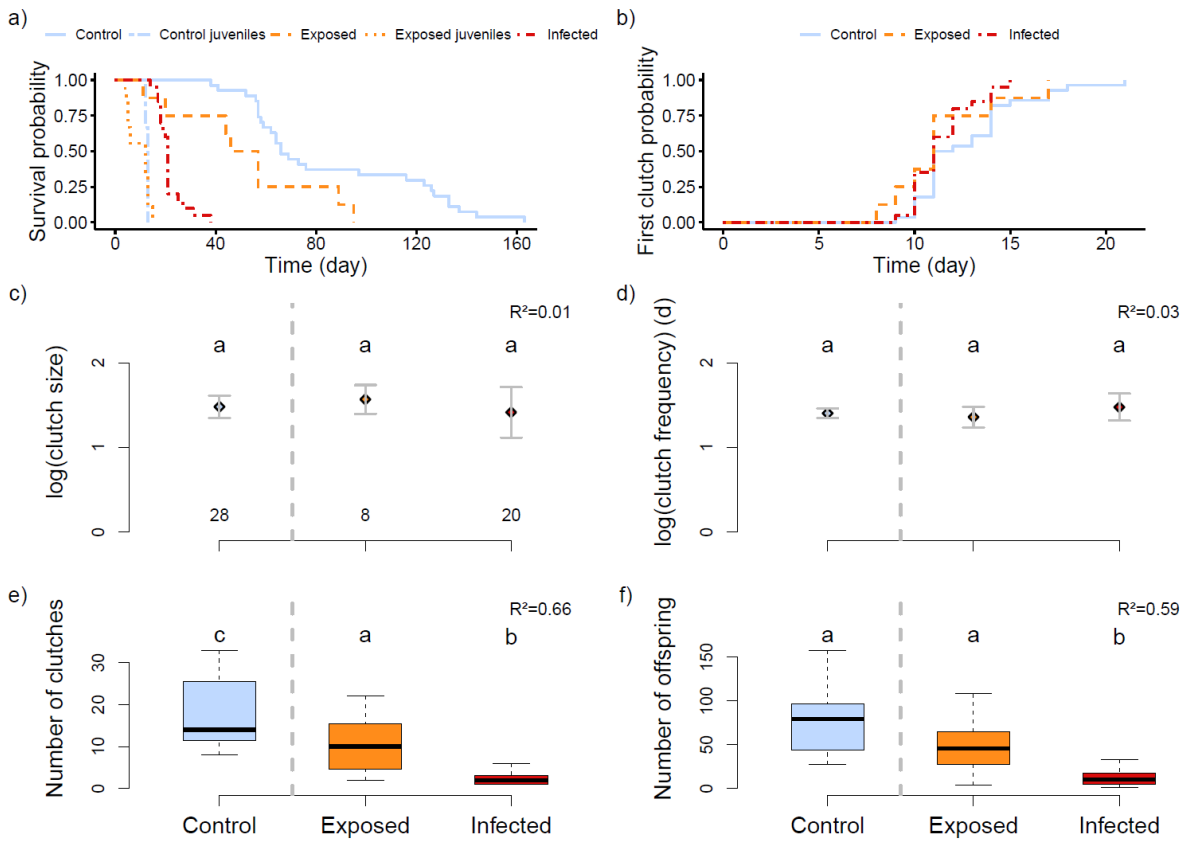


**Figure 1.** Effects of DIV-1 on host fecundity and survival. a) Survival of *D. magna* depending on infection status (healthy, exposed, or infected) and depending on whether or not they have offspring in their lifetime; b) age at maturity (first clutch); c) clutch size (log); d) clutch frequency (log);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

significantly reduced the total number of clutches (p-value = 0.001, Fig. 1e) with an intermediate value for exposed *D. magna*. Infection reduced total offspring production (p-value < 0.001, Fig. 1f), although exposed individuals did not show a reduction in total offspring compared to controls (p-value = 0.08).

For natural populations (Measure 2; Fig C1 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 Villette pond on 17 May, which had a lower fecundity for infected *D. magna* (p-value = 0.008).

### *3.2. Mobility (Measure 3)*

For experimentally infected *D. magna* (Fig. 2a, 2c, 2e, Appendix B), exposed individuals showed lower activity with a lower mean speed (p-value = 0.008) and a lower maximum speed

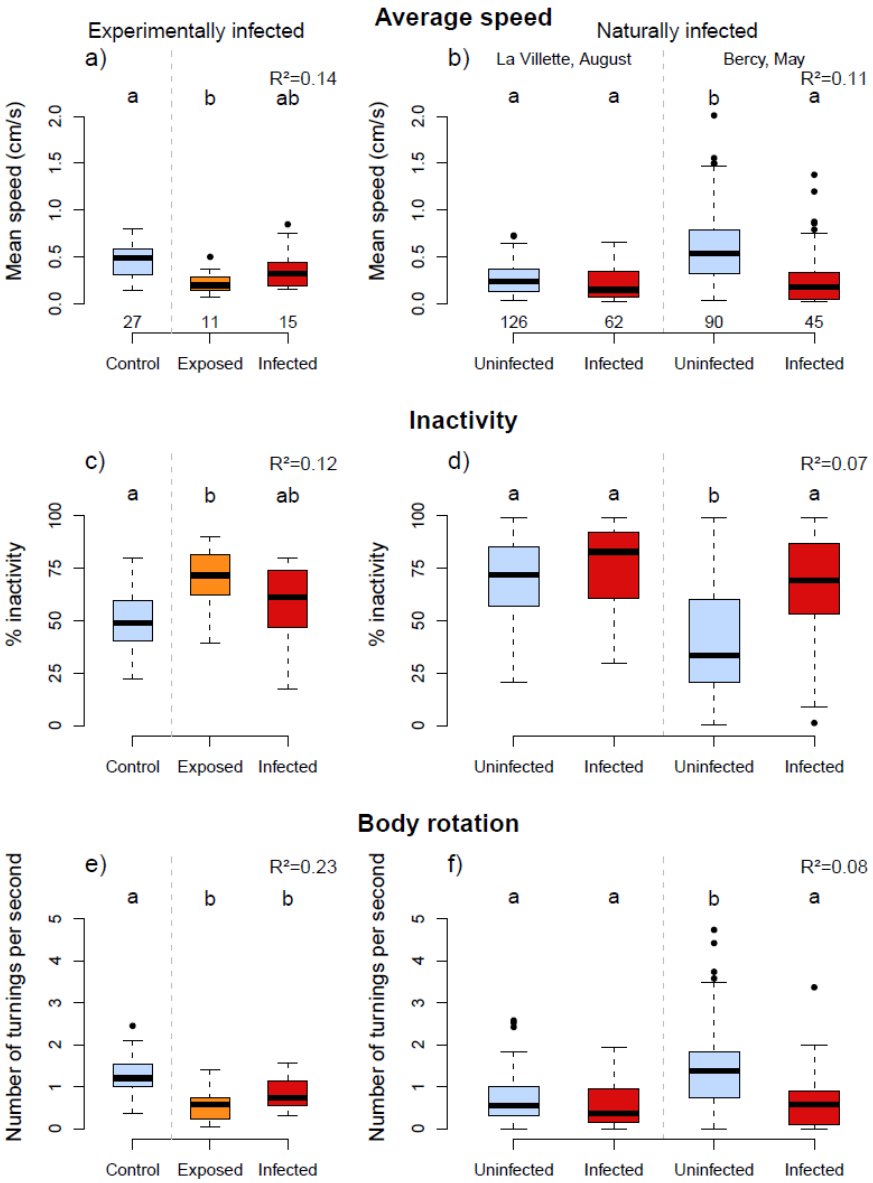


**Figure 2.** Effects of DIV-1 on host mobility on experimentally infected (left) and naturally infected (right) *D. magna*. a-b) Mean speed; c-d) proportion of inactive time; and e-f) number of turnings for *D. magna* with or without signs of DIV-1 infection. Note that the uninfected category aggregates 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.

(p-value = 0.006), and were more often inactive (p-value = 0.010) than control 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. 2b, 2d, 2f, Appendix B), 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).

### *3.3. Body size (Measure 4)*

We compared the size of healthy and infected *D. magna* (Fig. 3, Appendix B). For experimentally infected *D. magna* (same age), infected individuals were larger than controls (Fig. 3a, p-value = 0.043), while exposed *D. magna* had an intermediate size. For natural populations (Fig. 3b), 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, infected *D. magna* used for *Notonecta* sp. predation were larger than healthy individuals (p-value < 0.001).

### *3.4. Biochemical composition and energy value (Measure 5)*

We observed similar patterns in the two sampling ponds (p-values (status x pond) > 0.3, Table 1 and Fig. C2). Infected individuals of *D. magna* had the same amount of proteins as healthy brooding *D. magna* (p-value = 0.275 for La Villette) and had more proteins than healthy *D. magna* (p-value < 0.001 for La Villette). Infection and brooding did not change the amount

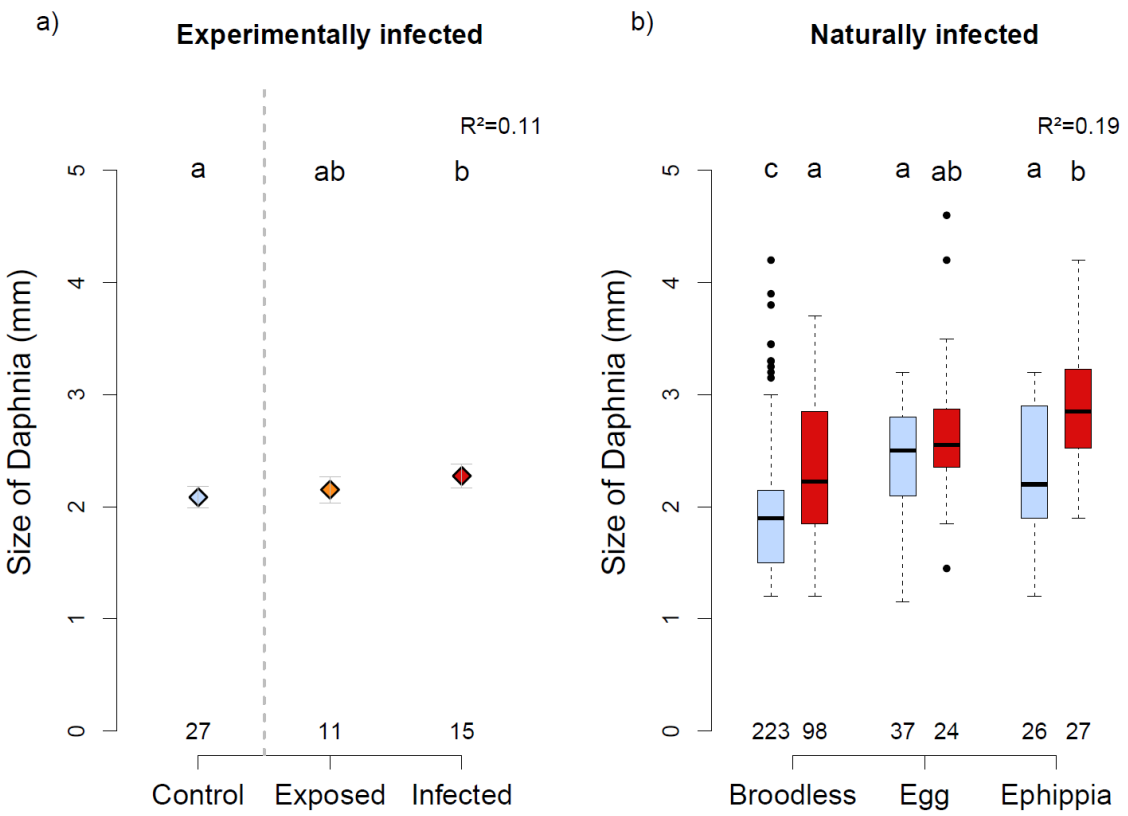


**Figure 3.** Effects of DIV-1 on host size on a) experimentally infected (healthy/control, exposed, infected); and b) naturally infected *D. magna* (broodless, with eggs, or with ephippia). Numbers are the numbers of *D. magna* for each category. The same letters indicate groups that are not significantly 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.

of triglycerides, while carbohydrates increased in the presence of eggs/embryos alone (p-values < 0.001). Finally, brooding and infected *D. magna* had a higher energy content (all p-values < 0.003).

### *3.5. Reflectance (Measure 6)*

From 280 to 850 nm, which includes the UV and visible domains, the reflectance of infected *D. magna* was higher (12.19 +/- 4.76%) than that of healthy *D. magna* (3.88 +/- 1.47%, Fig. C3). 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).

**Table 1.** 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) |

### *3.6. Vulnerability to predation (Measures 7 and 8)*

For both predator species (Measure 7 and Appendix A), the time elapsed before the capture of the first *D. magna* and between two consecutive captures did not differ between infected and

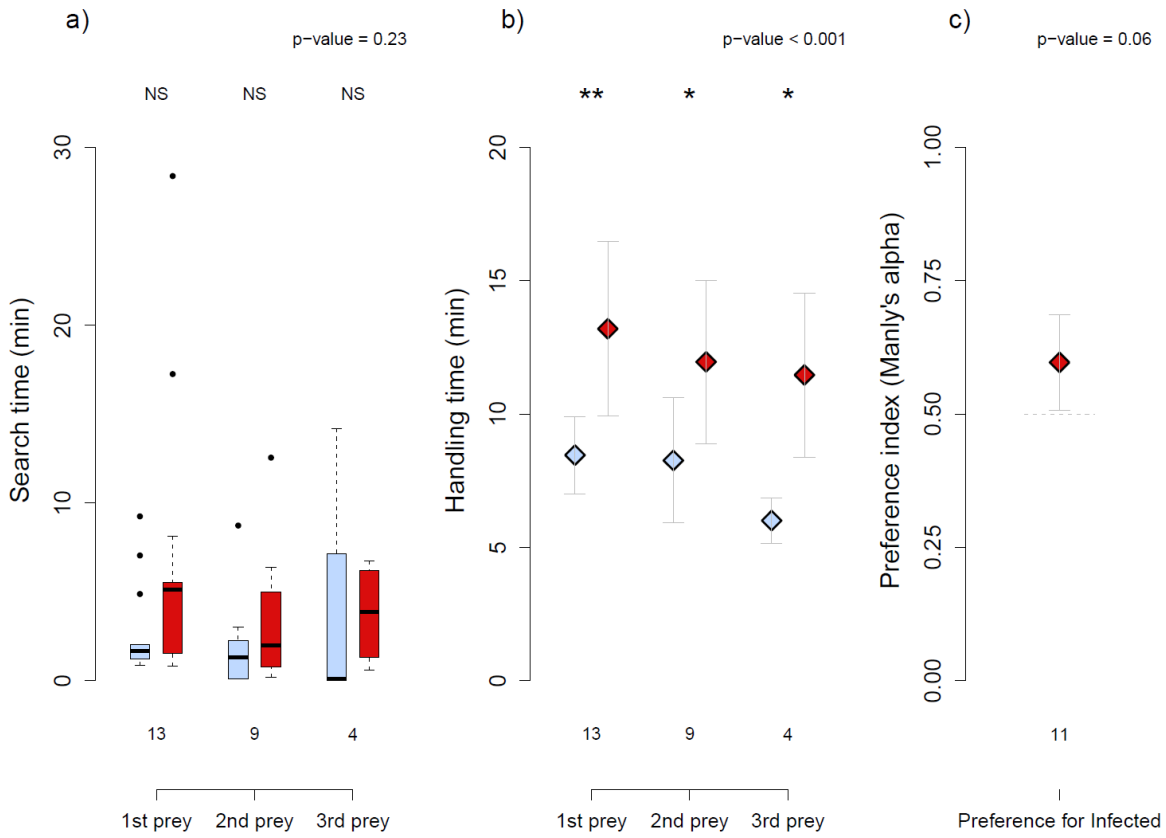


**Figure 4.** Effects of DIV-1 on vulnerability to predation. a) Search time and b) handling time by *Notonecta* sp., healthy (light blue) or infected (dark red), for the three prey; c) preference for infected *D. magna*. a,b) Statistics compare healthy versus infected prey: dot P < 0.1, *P < 0.05; **P < 0.01; 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.

uninfected *D. magna* (Fig. 4a, Fig. A1). However, the handling time by *Notonecta* was significantly longer when they preyed on infected *D. magna* (p-value <0.001 for all catches, Fig. 4b), which also tended to be preferred (Measure 8) over healthy *D. magna* (p-value = 0.061, Fig. 4c). Considering the values of handling time (Measure 7) and total energy content per *D. magna* (Measure 5), we approximated the profitability of healthy *D. magna* to be around 242/8=30 mJ/*Daphnia*, and that of infected *D. magna* around 449/12=37mJ/*Daphnia*.

# 4. Discussion

Parasites may affect their host in many ways, with potential repercussions for predators. Here, we investigated *D. magna* water fleas infected either naturally or experimentally with the iridovirus DIV-1 (Daphnia iridescent virus 1). We found that DIV-1 reduced the survival of water fleas, while effects on fecundity were not significant. We also noted that infection changed the phenotype of *Daphnia*, mainly by increasing the host size, coloration, and energy content. Such changes increased the profitability of infected individuals by 23%. Based on 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 without white coloration. Finally, we will highlight the complex consequences of parasitism on trophic links.

## *4.1. 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 to clutch size or clutch frequency, contrary to previous observations of lower fecundity in this studied 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 and 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 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 hypothesis that the virus progressively accumulates inside the host and ultimately leads to death.

Indirect effects of infections could be caused by less studied phenotypic alterations: body size, mobility, and reflectance. Infected individuals are larger; however, this effect is generally observed for infection by castrating parasites (Hall et al. 2007). Here, there is no effect on fecundity, meaning that an unknown physiological modification could explain it. A lower speed, generally associated with larger size (Dodson and Ramcharan, 1991), could be an explanation. 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 and

Strickler 1977), leading to the lower speed of this *D. magna* population. As a result, this would mask 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 and 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 and Ruck 1970). This is consistent with the tendency observed here in which *Notonecta* sp. prefers infected *D. magna*.

### *4.2. 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 lifecycle 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 and 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 and 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 30 and 37 mJ/s for uninfected and infected individuals, respectively, representing an increased profitability of 23%. The third parameter of the net energy gain, search time, is unchanged despite the modifications to host coloration and a possible reduction in mobility (also in the preliminary experiment with fish). Consequently, based on the 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, leading to negative repercussions on predator fitness (Flick et al. 2016) and 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 *Daphnia*'s predator while controlling DIV-1 prevalence to dampen parasite direct effects could be undertaken to explore the parasite indirect effects on predator diet.

### *4.3. 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. 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. We observe that this resistance results in the lower cost of lifespan (due to the infection, because the virus does not accumulate in the host) but also the greater cost of mobility (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 the host reaction to infection.

### *4.4. 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, 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 predator 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 the host density or parasite prevalence to separately consider the effects on host energy and host availability.

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 visible infections (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 this exposed class. Thus, in the continuity of this study, we question how this third class 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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# Data availability

Data are available on Zenodo. DOI: 10.5281/zenodo.6006618

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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 tendency for a lower search time for the first prey (Fig. A1, p-value = 0.081), we did not observe any effect for the second and third prey. 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).

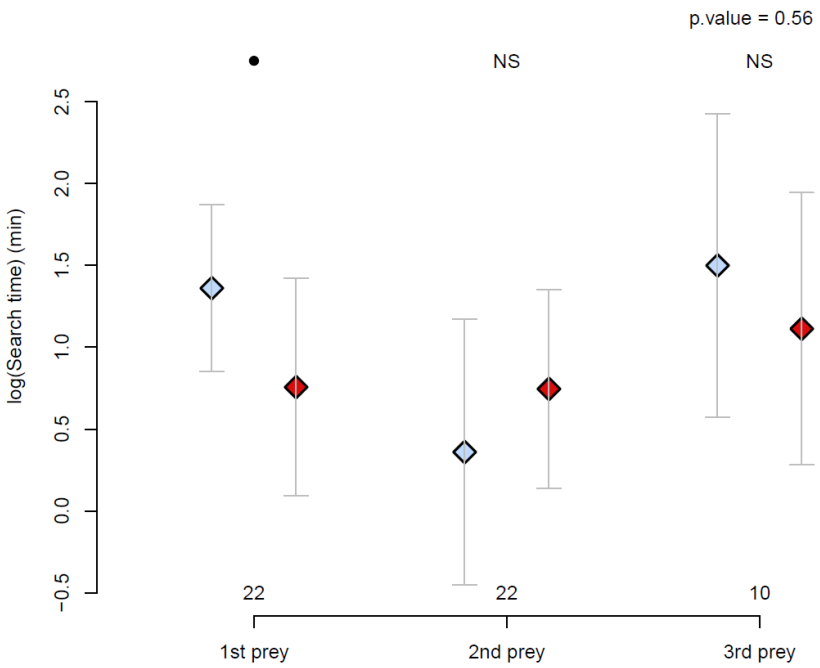


**Figure A1.** Effects of DIV-1 infection on vulnerability to predation by fish. Search time on healthy (light blue) or infected (dark red) prey for the three prey. Statistics compare healthy versus infected prey: dot P < 0.1, *P < 0.05; **P < 0.01; NS P>0.1. Dots represent the means and bars the 95%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

# Appendix B: Multiple Factorial Analysis of *Daphnia magna* traits

Because we performed various measures on the same individuals (experimentally infected *D. magna*), we used Multiple Factor Analysis (MFA). 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 (Fig. B1).

First, we can observe that the ellipses of the 95% interval confidence of the means do not overlap (Fig. B1b). Thus, there are significant differences between the Control, Exposed, and Infected *D. magna* but not sufficient to perform a classification that constitutes the three groups close to the infection status. The first two axes of the MFA allow us to separate the three *D.*

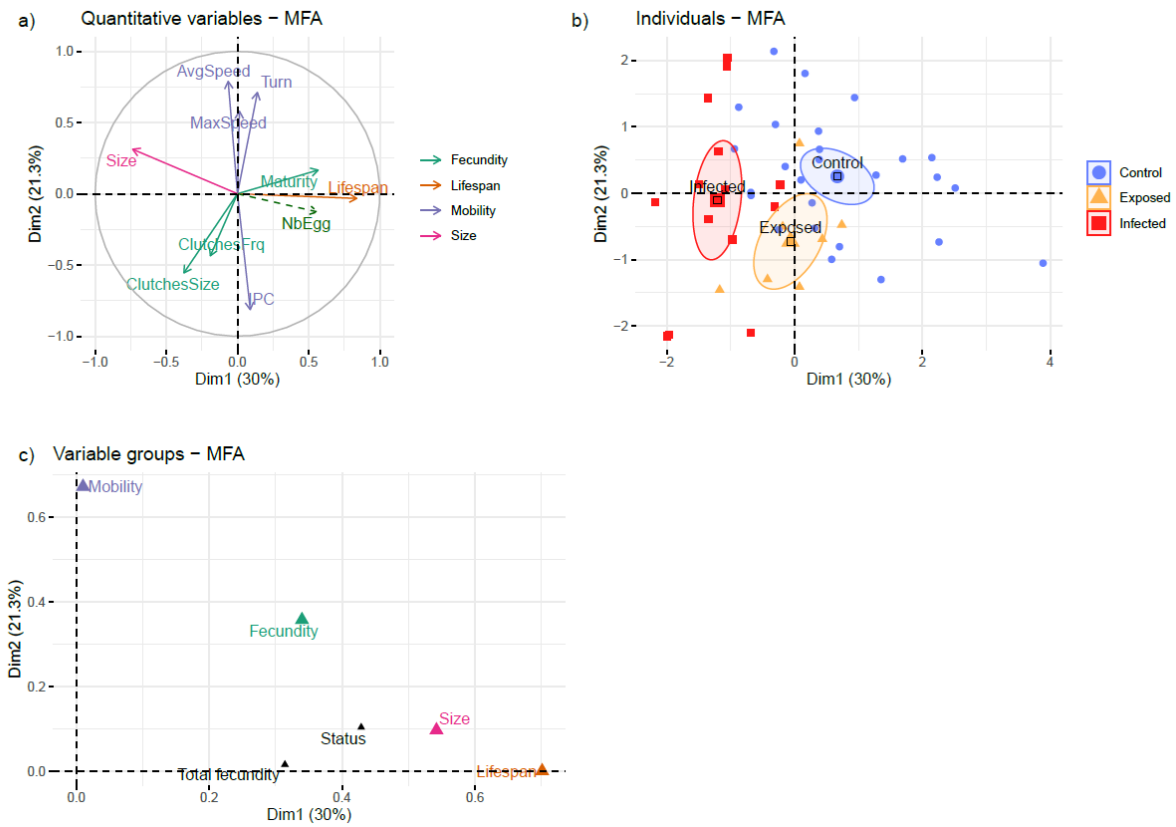


**Figure B1.** MFA on measurements of *D. magna* experimentally infected with DIV-1 for the two first dimensions. a) Quantitative variables grouped in four categories; note that total egg production (NbEgg) is a supplementary variable. b) Representation of individuals with ellipses for the 95% confidence interval. c) Representation of the group for the two dimensions.

*magna* groups. The first axis represents (Fig. B1a, B1c) Lifespan (positively correlated, p-value < 0.001) and Size (negatively correlated, p-value < 0.001). The second axis corresponds to the *D. magna* Mobility (negatively correlated, p-values < 0.001 for four parameters). Fecundity can be described by both axes: Age at maturity is positively correlated (p-value < 0.001) and Clutch Size is negatively correlated (p-value = 0.009) to first axis, Clutch Frequency (p-value = 0.022) and Clutch Size (p-value < 0.001) are negatively correlated to the second axis. Note that the total egg production is mainly correlated to the lifespan rather than the fecundity parameters, which shows that total egg production is highly impacted by *Daphnia* lifespan. 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 low mobility and intermediate lifespan. Infected individuals show a lower lifespan and larger size, with varying mobility. The first axis is therefore sufficient to separate Infected individuals from the others, although both the first and second axes are necessary to separate Control and Exposed individuals.

Thus, with this MFA, we conclude that considering these traits, the main effects of DIV-1 experimental infection are a reduced lifespan (Fig. 1a), which implies a reduction in total egg production (Fig. 1f) and an increase in individual size (Fig. 3b). We see that exposed individuals with an infection phenotype are quite distinct from Infected ones for the three aforementioned traits. However, they are also different from Control individuals due to the simultaneous lower lifespan and mobility.

# Appendix C: Supplementary figures and tables of statistics

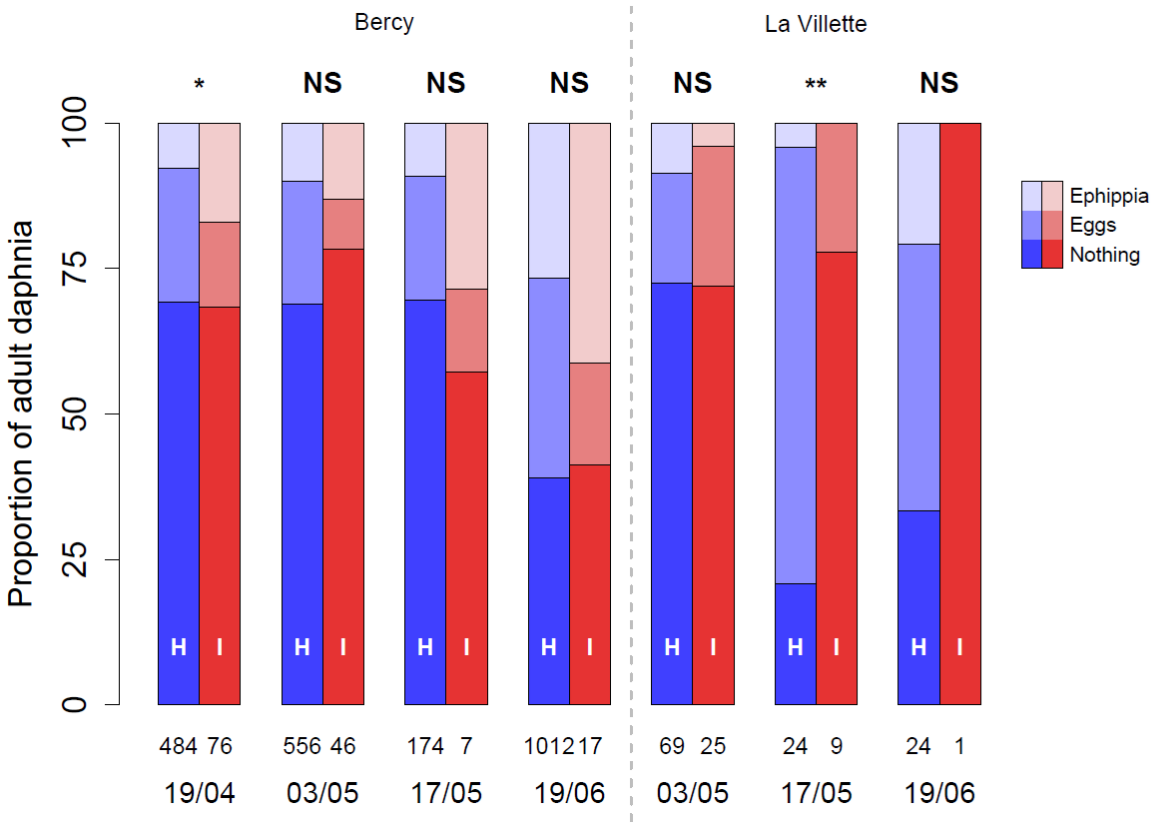


**Figure C1.** Proportion of adult *D. magna* without eggs, with eggs, or with ephippia depending on their infection status (healthy in blue, infected in red) in the two ponds for various dates. Numbers are the numbers of infected or uninfected *D. magna*. Statistics compare healthy versus infected prey: dot P < 0.1, *P < 0.05; **P < 0.01; NS P>0.1. See Table C2 for statistical values.

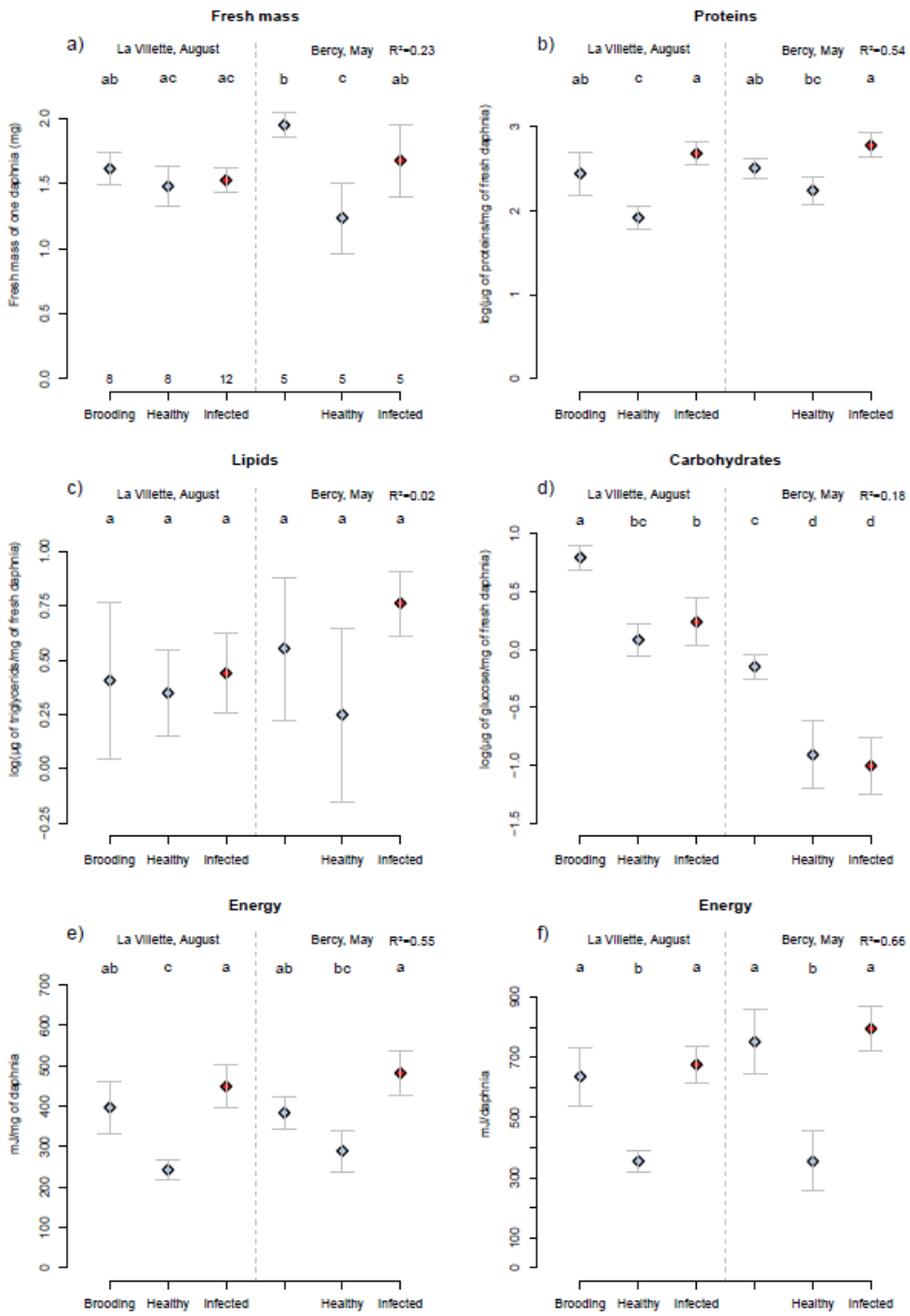


**Figure C2.** Energy content of *D. magna* for the two populations. a) Biomass, b) protein content, c) lipid content, d) carbohydrate content, e) energy (in mJ) by mg of *D. magna*, and f) energy (in mJ) by *D. magna*. Numbers in a) are the numbers in pools of 10 *D. magna* for each category. The same 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.

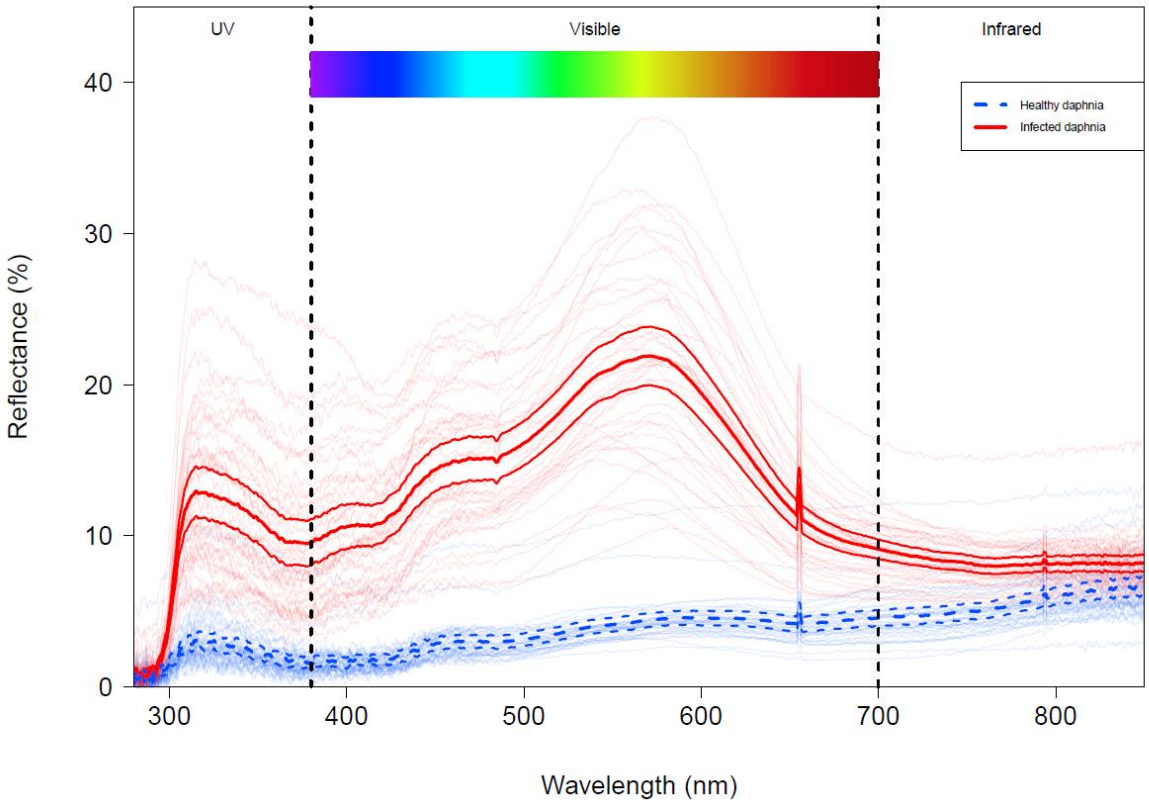


**Figure C3.** Effects of DIV-1 on reflectance between 280 and 850 nm. Blue (dashed) lines are healthy *D. magna* and red (solid) lines are infected *D. magna*. Highly visible lines are the mean and the lower and upper 95% confidence interval. Weakly visible lines correspond to all the measured *D. magna*. Note the two peaks due to the material (artefacts) around 660 nm and 790 nm. See Table C6 for statistical values.

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

| | | 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 |
| | $\chi^2$/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** | 0.91 | 1 | **< 0.001** | **< 0.001** |
| Control-Exposed | | **0.01** | **0.011** | 0.252 | 0.91 | 1 | **0.027** | 0.08 |
| Exposed-Infected | | **< 0.001** | **< 0.001** | 0.78 | 0.91 | 1 | **< 0.001** | **< 0.001** |

**Table C2.** Statistical results of DIV-1 effects on fecundity for naturally infected *D. magna* (Fig. C1)

| 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. 2)

| | | 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. 3)

| | | 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 |
| | $\chi^2$ | 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. C2, Table 1)

| | | 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. C3)

| | 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. 4 and A1)

| | | Search time | | Handling time | Preference |
|---|---|---|---|---|---|
| | | Fish | Notonecta | | |
| All catches | df | 53 | NA | NA | 10 |
| | t/v | 0.58677 | 127 | 6 | 2.1137 |
| | p-value | 0.56 | 0.227 | **< 0.001** | 0.061 |
| 1st catch | df | 21 | NA | 12 | |
| | t/v | 1.8357 | 27 | -4.312 | |
| | p-value | 0.081 | 0.216 | **0.001** | |
| 2nd catch | df | 21 | NA | -3.2928 | |
| | t/v | -0.77946 | 22 | 8 | |
| | p-value | 0.444 | 1 | **0.011** | |
| 3rd catch | df | 9 | NA | 3 | |
| | t/v | 0.58129 | 4 | -3.6364 | |
| | p-value | 0.575 | 0.875 | **0.036** | |