

Parasite-induced suppression of aggregation under predation risk in a freshwater amphipod Sociality of infected amphipods

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ABSTRACT

Recent findings suggest that grouping with conspecifics is part of the behavioural defences developed by amphipod crustaceans to face predation risk by fish. Amphipods commonly serve as intermediate hosts for trophically transmitted parasites. These parasites are known for their ability to alter intermediate host phenotype in a way that promotes predation by definitive hosts, where they reproduce. If aggregation in amphipods dilutes the risk to be preyed on by fish, then it may dilute the probability of transmission for the parasite using fish as definitive hosts. Using experimental infections, we tested whether infection with the fish acanthocephalan *Pomphorhynchus laevis* alters attraction to conspecifics in the amphipod intermediate host *Gammarus pulex*. We also measured *G. pulex*'s activity and reaction to light to detect potential links between changes in aggregation and changes in other behaviours. The attraction to conspecifics in the presence of predator cue, a behaviour found in uninfected gammarids, was cancelled by the infection, while phototaxis was reversed and activity unchanged. We found no correlation between the three behaviours in infected amphipods, while activity and aggregation were negatively correlated in uninfected individuals after the detection of predation cue. The physiological causes and the adaptive value of aggregation suppression are discussed in the context of a multidimensional manipulation.

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Aggregation is the tendency to gather with conspecific individuals. It can be permanent in social animals or temporary in other species. To assess the level of sociality, it is important to distinguish between aggregation as the result of the independent response of individuals to a common external stimulus (e.g. the patchy distribution of resources), or the mutual attraction of conspecifics that has evolved because of a selective advantage of grouping (Evans et al., 2007). Lowered predation risk through dilution or confusion effects is probably the most frequent benefit of aggregation, the main costs being a higher competition for resources and an enhanced risk of being parasitized (Krause and Ruxton, 2002). Aggregation can be generated by mating events, the size and composition of aggregates varying with species and mating systems (Elmen and Oring, 1977). Aggregation is well documented in vertebrates (Krause and Ruxton, 2002) and to a lesser extent in marine crustaceans where gregarious behaviours are observed during mating, moulting or overwintering (Ritz, 1984; DeGoursey and Stewart, 1985;

Zimmer-Faust et al., 1985; Zimmer-Faust and Spanier, 1987). In freshwater invertebrates, aggregation was investigated only very recently. It has been shown that the odour of a fish predator triggers conspecific attraction in the freshwater amphipod *Gammarus pulex*, whereas no significant aggregation is observed in the absence of predation risk (Kullmann et al., 2008; Thünken et al., 2010).

Amphipods are common intermediate hosts of acanthocephalans, a phylum of trophically transmitted parasites displaying a multiple host life cycle. Parasite larvae develop in invertebrates while adults mature and reproduce sexually in vertebrate definitive hosts (Crompton and Nickol, 1985). Eggs are released in the environment with faeces and ingested by invertebrates. Because the predation event between the successive hosts is associated with high mortality rates (Dobson, 1988), trophically transmitted parasites such as acanthocephalans are under strong selective pressure to evolve strategies making intermediate hosts more susceptible to predation. Infection with trophically transmitted parasites is most often associated with phenotypic alterations in the intermediate host, with changes in appearance, physiology or behaviour (Moore, 2002). These so-called "host manipulations" have been considered as an adaptive extended phenotype of the parasites, favoured by natural selection (Thomas et al., 2005). The ability to manipulate host phenotype is thought to be an ancestral character among acanthocephalans (Moore, 1984), which commonly rely on inhibiting or

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reversing the anti-predator responses of their intermediate host to favour transmission. For instance, amphipods infected with cystacanths of the fish acanthocephalan *Pomphorhynchus laevis* are attracted instead of being repulsed by fish odour (Baldauf et al., 2007; Kaldonski et al., 2007). They also show an increased drift and an altered reaction to light (reversed phototaxis) compared to uninfected individuals that stay motionless in refuges to avoid predation (Bauer et al., 2000; Cézilly et al., 2000; Kaldonski et al., 2007; Lagrue et al., 2007). As a result, fish predators show a preference for amphipods infected by *P. laevis*, both under laboratory conditions (Kaldonski et al., 2007; Dianne et al., 2011) and in the field, where the proportion of infected *G. pulex* in the stomach of bullheads (*Cottus gobio*), one of the definitive hosts of *P. laevis*, was found to be 26.3–28.3 times higher than the prevalence in the benthos (Lagrue et al., 2007).

Although the adaptive value of aggregation has not been formally experimentally tested in amphipods, it is assumed to decrease predation risk by fish (Kullmann et al., 2008; Thünken et al., 2010), and can thus be seen as another anti-predatory response among the battery of behavioural defences they developed to face predation. From the parasite's perspective, the aggregation of infected intermediate hosts with (uninfected) conspecifics under predation risk by a definitive host dilutes its chance of being transmitted. In other words, the parasite may benefit from inhibiting aggregation in its intermediate host. Here, we investigated whether the infection with *P. laevis* influences the aggregative behaviour of *G. pulex*. We measured its tendency to stay close to conspecifics in the presence or absence of chemical cues from a fish definitive host. We also estimated amphipods' activity during aggregation experiments to investigate the link between grouping and activity. We finally measured reaction to light under the two predation regimes as a control of parasite-induced behavioural changes, since *P. laevis*-infected amphipods are known to be attracted to light whereas uninfected conspecifics are photophobic (Bauer et al., 2000; Cézilly et al., 2000; Franceschi et al., 2008). In uninfected amphipods, we expected to observe a significant tendency to group with conspecifics under predation risk only (Kullmann et al., 2008). In infected amphipods, we expected either the absence of such anti-predatory response whatever the predation regime, or, if infection reverses this putative anti-predatory response, a lower grouping under predation risk.

1. Methods

1.1. Experimental infections

1.1.1. Amphipod and parasite sampling

In February 2010, amphipods were collected with a 500- μ m mesh pond net from a tributary of the Suzon River (Côte d'Or, France, 47°24'N and 4°52'E) where *P. laevis* has never been found (L. Bollache, unpublished data). At this site, amphipods may harbour the larval cestode *Cyathocephalus truncatus* (Franceschi et al., 2007) and the muscle wasting microsporidian *Pleistophora mulleri* (K. Monceau and T. Rigaud, unpublished data). To avoid the confounding effects associated with multiple infections, individuals infected by these symptomatic parasites were discarded during sampling. Amphipods may also harbour asymptomatic, vertically transmitted microsporidia that potentially confound acanthocephalan-induced behavioural changes (Haine et al., 2005). These parasites have never been found in the *G. pulex* population we used for controlled infections (Franceschi et al., 2008 and T. Rigaud, unpublished data). Because female amphipods suffer higher mortality than males during controlled infections (Franceschi et al., 2008), we selected only intermediate-sized males for experiments (9 ± 1 mm in total length, from the tip of the rostrum to the base of the telson) based on

sexual dimorphism with males displaying larger gnathopods than females (Médoc et al., 2006). In the laboratory, amphipods were acclimatized for 10 days in 30-L housing tanks containing a layer of gravel, macrophytes, and filled with aerated site water at a temperature of 15 ± 1 °C, under a 12:12 h light/dark cycle. Amphipods were fed with conditioned elm leaves at satiation.

Following the procedure of Franceschi et al. (2008), parasite eggs were taken from the female parasites of naturally infected chubs (*Leuciscus cephalus*) caught by electrofishing (Dream Electronique Society, France) in the Vouge River (Côte d'Or, 47°08'N and 5°10'E). In the laboratory, chubs were anaesthetised and euthanatised with clove oil (90% eugenol), decapitated and dissected, looking for adult acanthocephalans fixed inside the digestive tract. To confirm death visually before decapitation, we watched the opercular movements once the fish turned on its side and removed the fish fifteen minutes after the last opercular movement. Eggs were obtained by dissecting female parasites, whose genotype was assigned to that of *P. laevis sensu stricto* by a diagnostic PCR test based on the length of the ITS amplification products (see Perrot-Minnot, 2004 and Franceschi et al., 2008 for details). The clutches of 12 *P. laevis* females from 4 distinct chubs were mixed in microtubes filled with 400 μ L of water and the concentration of mature eggs [i.e. eggs with the acanthor larval stage inside (Crompton and Nickol, 1985)] was estimated by averaging the counts made under a microscope (Nikon E 600) in 10 samples of 1 μ L.

1.1.2. Infection procedure

Amphipods were placed in glass dishes (6 cm diameter) with aerated river water and fed with one 1-cm² dry elm leaf on which the egg suspension was deposited so as to give approximately 100 mature eggs per amphipod [a dose that provides satisfactory prevalence and favours monoinfections according to Franceschi et al. (2008)]. After a 48-h exposure, amphipods were returned to housing tanks and inspected daily under a binocular microscope from the sixth week to detect cystacanths, which are visible through their cuticle. The cystacanth stage is the developmental stage from which the parasite is infective towards the definitive host and starts manipulation to promote transmission (Dianne et al., 2011). Control amphipods (i.e. uninfected amphipods) were processed similarly but fed with leaves free of parasite eggs.

1.2. Behavioural experiments

1.2.1. Aggregation and activity

The experimental set-up was identical to the one used by Kullmann et al. (2008) and consisted of a plastic aquarium (30 cm \times 20 cm \times 20 cm height) filled with 3 L of aerated water from the amphipod collection site, divided into three equal-sized compartments (10 cm \times 20 cm) by two lines drawn on the bottom. The middle compartment served as neutral zone whereas the outer compartments served as choice zones equipped with a tea ball (35 mm diameter and 0.2 mm mesh metal grid) hung in the middle 1 cm above the bottom. The metal grid allowed chemical exchange with the surrounding water while visual and vibrational contacts were limited. Ten randomly chosen amphipods: 5 uninfected and 5 infected, were placed into one tea ball while the other remained empty. We used a mix of infected and uninfected amphipods to avoid a potential effect of infection status on conspecific attraction in the amphipod under test. Ten minutes after the introduction of tea balls, one *G. pulex* (either infected or control uninfected) was put into a translucent plastic cylinder (35 mm diameter) placed in the middle of the neutral zone. After a 2-min acclimatization period, the cylinder was lifted and we recorded the amphipod location every 30 s over 5 min by direct observations from above the aquarium. At each observation (10 observations per trial), an

aggregation score was assigned depending on the location: “0” for the neutral compartment, “−1” for the choice compartment without amphipods and “+1” for the choice compartment with amphipods. At the end of each trial, summed scores ranged from “−10” (always in the choice compartment without conspecifics) to “10” (always in the choice compartment with conspecifics). To estimate amphipod’s activity, we determined how many times the amphipod crossed the lines dividing the experimental unit based on its location from one observation to the next. For instance, if the amphipod was recorded in the neutral zone at 30 s and in one of the two choice compartments at 1 min, then we considered that it crossed one line. An activity score, corresponding to the total number of lines crossed over the 5 min and ranging from “0” (always in the same zone) to “18” (always moving between the two choice compartments), was assigned to each amphipod. Compared to continuous monitoring, this design probably underestimates the number of movements but provides an estimation of the amphipod’s activity level during the aggregation experiment.

1.2.2. Reaction to light

The experimental set-up was similar to the one used by Perrot-Minnot (2004) and consisted of a horizontal glass tube (25 mm diameter and 15 cm long) closed at each end and filled with aerated water from the amphipod collection site at 15 ± 1 °C. One half of the tube was painted in black to ensure complete opacity (dark zone) while the other was left translucent (light zone). A hole (5 mm diameter) was drilled in the middle to allow the introduction of a single amphipod. Lighting was provided by a 60 W fluorescent tube mounted 40 cm above the tube. After the introduction of a single amphipod and a 2-minute acclimatization period, its position was recorded every 30 s during 5 min and scored as “0” or “1” for dark and light zones, respectively. At the end of each trial, summed scores ranged from “0” (always in the dark zone) to “11” (always in the light zone).

1.2.3. Experimental procedure

All experiments were performed under two water conditions: “control water” (i.e. aerated water from the amphipod collection site) and “scented water” to mimic predation risk. To obtain scented water, 48 trout [*Salmo trutta fario*, a definitive host of *P. laevis* (Kennedy et al., 1978), 10–14 cm in fork length] from a commercial fishery were placed for 24 h per groups of 8 in 6 tanks (46 cm × 34 × 38 cm height) filled with 50 L of aerated site water. Trout were preferred to chubs to scent water since they were used in previous studies to obtain fish predation cues (Dianne et al., 2011), which allows comparison of results. Furthermore, because trout come from a commercial fishery, it is easy to standardise fish size and predation cues between studies. As a food source, each group of trout received 40 uninfected amphipods that were all consumed at the end of the 24-h exposure, which represented 0.8 predation event per litre. This process was expected to provide a predation signal close to the one observed under natural conditions since it includes both the chemical cues released by injured prey and predator (Wudkevich et al., 1997; Abjörnsson et al., 2000; Wisenden, 2000; Wisenden et al., 2001). All amphipods experienced each behavioural test under the two water conditions successively (paired data, $N = 44$ and 45 for uninfected and infected amphipods, respectively). After the experiments, they were anaesthetised with clove oil, sacrificed by thermal shock, measured with a digital calliper (total length to the nearest 0.1 mm) and dissected to confirm their infection status. The experimental setups were rinsed with tap water after each trial. To avoid a temporal effect, the order of the two behavioural tests and the two water conditions was inverted between each amphipod. To avoid a side effect, the

position of light and dark zones and the two choice compartments were inverted between each amphipod.

1.3. Statistics

The difference in body length between infected and uninfected amphipods was tested with a Student’s *t*-test and found not significant ($t_{87} = -0.41$ and $P = 0.68$). Since the behavioural data on aggregation and activity met the homocedasticity assumption, the effects of infection status, amphipod body length and water conditions on aggregation and activity scores were analysed using an ANOVA for repeated measures. Conversely, we used non-parametric statistics to analyse the data on phototaxis since they were not normally distributed and could not be transformed satisfactorily. The between-infection status difference was tested with a Mann–Whitney’s *U*-test and the between-water condition difference with a Wilcoxon’s signed rank test. The correlations between phototaxis score and amphipod body length, and between the three behavioural traits recorded were investigated using Spearman’s correlation tests with Bonferroni’s correction. All statistical analyses were 2-sided with a 5% significance level and performed with R version 2.7.2 software (R Foundation for Statistical Computing) and JMP 6.0 software (SAS Institute).

2. Results

2.1. Aggregation and activity

All the amphipods placed into the tea ball were alive at the end of the experiments, which indicates the absence of cannibalism. Amphipod size never influenced aggregation scores, neither alone nor in interaction with other factors. Conversely, aggregation scores were significantly influenced by both infection status and water condition, and, most importantly, by the interaction between these two factors (Table 1a). *P. laevis*-infected individuals showed similar weak aggregation scores whatever the water condition, while uninfected *G. pulex* showed a weak aggregation score in control water and a high score in scented water (Fig. 1a). The aggregation score was significantly different from a score of 0 (indicating neutrality) only for uninfected *G. pulex* in scented water (Wilcoxon’s signed-rank test, $N = 44$, $P < 0.0001$). Activity scores were significantly influenced by water condition but not by amphipod size, infection status nor the interaction between infection status and water condition (Table 1b). Both infected and uninfected amphipods showed decreased activity scores in scented water compared to control water (Fig. 1b).

Table 1

ANOVA for repeated measures analysing the effect of infection status (uninfected or infected with the fish acanthocephalan *Pomphorhynchus laevis*) and water condition (control or scented water) on aggregation (a) and activity (b) scores in the freshwater amphipod *Gammarus pulex*. Amphipod size and the associated interactions had no significant effect and were removed from the models.

Factor	Exact F	NumDF	DenDF	P
(a)				
Between subjects				
Infection	18.32	1	87	<0.0001
Within subjects				
Water	8.24	1	87	0.005
Water * Infection	17.97	1	87	<0.0001
(b)				
Between subjects				
Infection	3.16	1	87	0.08
Within subjects				
Water	14.60	1	87	0.0002
Water * Infection	0.14	1	87	0.71

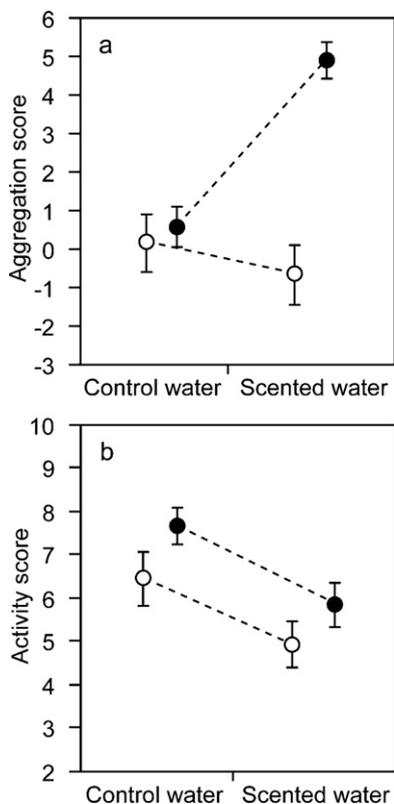


Fig. 1. Aggregation (a) and activity (b) scores (means \pm SEM) of the freshwater amphipod *Gammarus pulex* either uninfected (black dots, $N=44$) or infected (white dots, $N=45$) with the fish acanthocephalan *Pomphorhynchus laevis*. Aggregation and activity were measured in the absence of predation risk (control water) and under the scent of fish predation (scented water).

2.2. Reaction to light

There was no significant correlation between amphipod size and phototaxis score (Spearman's correlation tests with Bonferroni's correction, all $P > 0.0125$). Infected amphipods showed significantly higher phototaxis scores than uninfected amphipods in both water conditions (Mann–Whitney's U -tests, in control water: $U=200$, $N_1=44$, $N_2=45$ and $P < 0.0001$, in scented water: $U=154$, $N_1=44$, $N_2=45$ and $P < 0.0001$, Fig. 2). The water condition did not affect the phototaxis score in uninfected *G. pulex* (Wilcoxon's signed rank test: $T=1017.5$, $N=44$ and $P=0.49$), which remained close to zero,

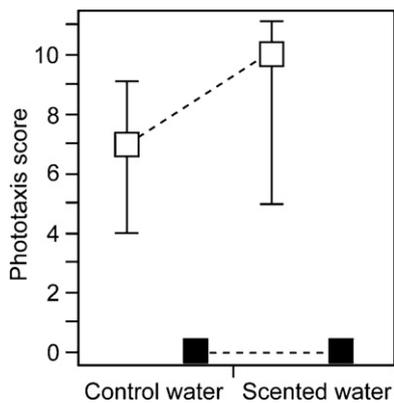


Fig. 2. Phototaxis scores (medians and interquartile ranges) of the freshwater amphipod *Gammarus pulex* either uninfected (black squares, $N=44$) or infected (white squares, $N=45$) with the fish acanthocephalan *Pomphorhynchus laevis*. Phototaxis was measured in the absence of predation risk (control water) and under the scent of fish predation (scented water).

whereas infected amphipods displayed a higher phototaxis score in scented water than in control water (Wilcoxon's signed rank test: $T=749$, $N=45$ and $P=0.03$, Fig. 2).

2.3. Correlations between behavioural traits

In infected amphipods, the correlations between scores were never significant, neither under predation risk nor in control water (Table 2). In uninfected amphipods, however, a negative correlation between activity and aggregation scores was found significant in scented water, the most active individuals being also the less aggregated (Table 2 and Fig. 3a). Reactions to predation cue in terms of activity and aggregation were also negatively correlated: the animals reducing strongly their activity were those that also increased strongly their aggregation (Table 2 and Fig. 3b).

3. Discussion

We used controlled infections to test under laboratory conditions whether infection with the fish acanthocephalan *P. laevis* alters conspecific attraction behaviour under predation risk in the freshwater amphipod *G. pulex*. We also estimated the activity level of amphipods during aggregation experiments and measured their reaction to light (phototaxis), a well documented *P. laevis*-induced alteration that we used as a control for manipulation. Although activity was not changed by infection, aggregation and phototaxis were altered by the presence of *P. laevis*.

We first found, in agreement with previous results (Kullmann et al., 2008), that uninfected amphipods were attracted by conspecifics in fish-conditioned water but did not aggregate in the

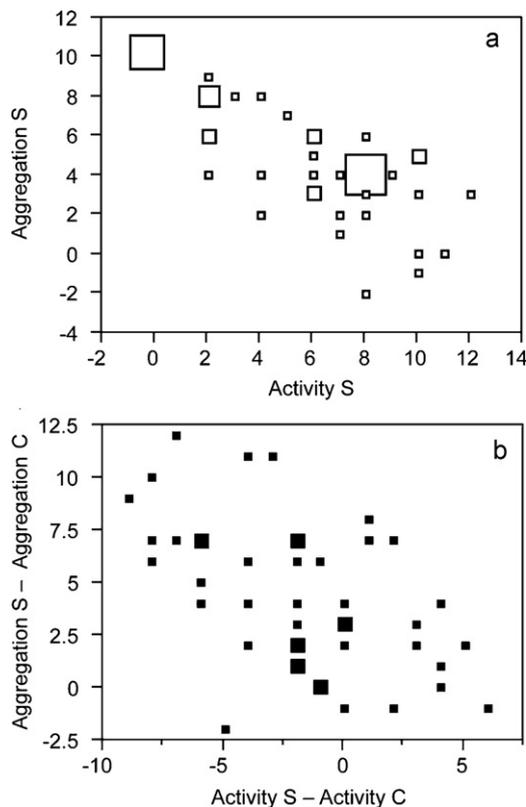


Fig. 3. Non-parametric correlations between individual aggregation and activity scores (a) measured in water scented by fish predation (S) in the freshwater amphipod *Gammarus pulex* infected with the fish acanthocephalan *Pomphorhynchus laevis* ($N=45$). The correlation is also given for individual scores from which the respective scores obtained in control water (C) were subtracted (b). Square sizes indicate overlapping data ranging from 1 to 6.

Table 2

Non-parametric correlations between individual phototaxis, aggregation and activity scores in the freshwater amphipod *Gammarus pulex* either uninfected or infected with the fish acanthocephalan *Pomphorhynchus laevis*. Scores were measured in control water (C) or in water scented by fish predation (S). Correlations are also given for individual reactions of amphipods to predation cue, by subtracting the scores obtained under scented water from those obtained under control water. Values in bold are those remaining significant after Bonferroni's correction.

Variable 1	Variable 2	Spearman's ρ	P
Infected amphipods			
Phototaxis C	Aggregation C	0.08	0.58
Activity C	Aggregation C	-0.06	0.70
Activity C	Phototaxis C	0.31	0.04
Phototaxis S	Aggregation S	-0.19	0.22
Activity S	Aggregation S	0.09	0.54
Activity S	Phototaxis S	-0.03	0.82
Phototaxis S – Phototaxis C	Aggregation S – Aggregation C	-0.05	0.74
Activity S – Activity C	Aggregation S – Aggregation C	0.28	0.06
Activity S – Activity C	Phototaxis S – Phototaxis C	0.02	0.89
Uninfected amphipods			
Phototaxis C	Aggregation C	0.11	0.48
Activity C	Aggregation C	-0.26	0.09
Activity C	Phototaxis C	-0.02	0.88
Phototaxis S	Aggregation S	0.18	0.24
Activity S	Aggregation S	-0.73	<0.0001
Activity S	Phototaxis S	-0.18	0.23
Phototaxis S – Phototaxis C	Aggregation S – Aggregation C	-0.11	0.48
Activity S – Activity C	Aggregation S – Aggregation C	-0.52	0.0003
Activity S – Activity C	Phototaxis S – Phototaxis C	0.27	0.07

absence of predation cue. As predicted based on the parasite's interest in terms of transmission, infected amphipods did not exhibit any tendency to aggregate whatever the cue regime (control or water scented with predation odours). Comparable results were observed with the cestode *Schistocephalus solidus*, a parasite using copepods as first intermediate hosts and sticklebacks as second intermediate hosts (Jakobsen and Wedekind, 1998). During choice experiments and under the threat of fish predation, uninfected copepods preferred the side with the odour of conspecifics whereas infected individuals avoided it. Because there is no between-amphipods transmission of *P. laevis*, aggregation suppression is unlikely to be a host strategy for protecting against infection. If aggregation in amphipods significantly decreases predation risk by fish, then inhibiting this anti-predatory response could be advantageous for the parasite by increasing the probability of a trophic transmission event between intermediate and definitive hosts.

However, the adaptive significance of aggregation suppression regarding parasite transmission might be questionable because it could be a side effect of other *P. laevis*-induced alterations such as changes in activity or reaction to light. We found no evidence for a change in activity induced by infection. In addition, no correlation was found between activity and aggregation in infected *G. pulex*, whereas for uninfected amphipods the more they reduced activity, the more they aggregated. Infected amphipods as well reduced their activity under predation risk but this behavioural reaction was disconnected from attraction to conspecifics. Decreasing activity is a common behavioural defence among invertebrates and particularly in amphipods (Andersson et al., 1986; Bollache et al., 2006), and our findings suggest that the correlation between activity and aggregation is likely to be part of the overall anti-predatory response displayed by *G. pulex* to face fish predation. *P. laevis* infection therefore disrupts incompletely the anti-predatory response in *G. pulex* (aggregation is cancelled but not the decrease in activity). Taken together, these findings support the idea that aggregation suppression is not a side effect of *P. laevis*-altered activity.

There was no significant correlation between aggregation and phototaxis, whatever the infection status of amphipods. Notably, the increase in phototaxis score from control to scented water in

infected amphipods was not linked with any aggregation or activity characteristics, or changes in these behaviours according to predation cue. Although the absence of correlation does not mean formally the absence of a link between the behavioural traits, it is reasonable to consider that phototaxis is therefore weakly linked to the two other behavioural traits, even in infected *G. pulex*. This last remark deals with the multidimensional aspect of parasite-induced manipulation. Multidimensionality means that infection with a single parasite generally affects more than one trait (i.e. dimension) in the intermediate host phenotype (Cézilly and Perrot-Minnot, 2005, 2010; Thomas et al., 2010). A multidimensional manipulation can be seen either as a set of alterations that all find their origin in a single physiological process, for instance if the parasite secretes only one single compound resulting in a cascade of effects and leading to an "infection syndrome" (Cézilly and Perrot-Minnot, 2010), or conversely as the sum of functionally independent alterations (Thomas et al., 2010). As already mentioned, we found no significant correlation between the three behaviours investigated in *P. laevis*-infected *G. pulex*, suggesting that the parasite's influence depends on the phenotypic trait and that manipulation does not consist simply to turn off the host's anti-predatory responses. Even if the relationship between two behaviours is probably much more complex than a simple correlation (Cézilly et al., 2010), we found no evidence for a coherent "infection syndrome". This is consistent with the findings of Benesh et al. (2008), who found no correlation in isopods between abdominal coloration and hiding, two traits modified by infection with *Acanthocephalus lucii*.

The increased attraction to light under predation cue in *P. laevis*-infected *G. pulex* deserves to be highlighted, because it can be seen as a contradiction with some previous results. Cystacanth-infected amphipods slightly increase the use of refuges in the presence of predation cue (even if they use refuges less intensively than uninfected animals, Kaldonski et al., 2007; Dianne et al., 2011). Refuge use is probably a complex behaviour. However, we could have expected that attraction to light was a component of this behaviour, because refuges are dark compared to open water. The discrepancy between our result on increased phototaxis and previous results on increased refuge use under predation risk suggests, again, that these two behavioural responses are not strictly linked,

or at least that the parasite could disrupt them in a rather independent way. A valuable perspective to test whether or not distinct phenotypic changes share a single proximal origin is to study the timing of manipulation. If they occur successively during parasite's development, then they are likely to be independent, with different physiological bases (Médoc and Beisel, 2011).

From a proximal point of view, *P. laevis* infection is correlated with changes in the brain serotonergic activity of amphipods (Tain et al., 2006), which could affect odour-dependent behaviours (Kloppenburg et al., 1999). So infection may cancel *G. pulex*'s olfactory assessment of predation risk by fish, which would explain why infected amphipods did not adjust their behaviour in conditioned water. This is, however, improbable since in our experiments infected amphipods responded to fish cue by decreasing activity and increasing attraction to light. They are thus still capable of assessing predation risk by fish through the perception of chemical signals. Furthermore, *P. laevis*-infected amphipods are known to be attracted by fish cues when uninfected amphipods avoid them (odour of perch: Baldauf et al., 2007, odour of bullhead: Kaldonski et al., 2007). An alternative hypothesis that could explain the disappearance of aggregation after *P. laevis* infection may be the inability to perceive the olfactory cues released by conspecifics, as it was proposed to explain the reduced ability to initiate a precopula in *P. laevis*-infected *G. pulex* (Bollache et al., 2001). However, this hypothesis is not consistent with the results found in amphipods infected by another acanthocephalan, *Polymorphus minutus*. In this case, the pairing success of the host is also strongly reduced but they are still attracted by conspecifics in fish-conditioned water (Thünken et al., 2010). So *P. minutus*-infected amphipods can perceive the chemical "signature" of conspecifics. Comparing these two parasitic infections is nevertheless not straightforward since *P. laevis* and *P. minutus* behavioural alterations are thought to rely on distinct physiological bases (Tain et al., 2006).

Our study provides additional evidence that grouping in amphipods is a behavioural response to predation risk. We also found that cancelling this behavioural response is an independent component of the multidimensional manipulation by *P. laevis*. Now, additional experiments are needed to test the adaptive value of aggregation suppression in terms of parasite transmission. This means using specifically designed experimental set-ups to disentangle the multidimensional manipulation and to measure the respective contribution of each dimension to the increased susceptibility to definitive host predation (see Kaldonski et al., 2009 for an example). From a more general perspective, social interactions in freshwater invertebrates need further investigations. In this respect, our study suggests that grouping in amphipods cannot be appraised as grouping in vertebrates such as fish. While the latter form shoals under predation risk, amphipods first hide and reduce their activity to then prefer the refuges with conspecifics.

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