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Individual behavioural responses of an intermediate host to a manipulative acanthocephalan parasite and the effects of intra-specific parasite competition

Timo Thünken[1], Simon Vitt[1], Sebastian A. Baldauf[1], Tina Jung[1] and Joachim G. Frommen[2]

[1]*Institute for Evolutionary Biology and Ecology, University of Bonn, Bonn, Germany and* [2]*Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Hinterkappelen, Switzerland*

ABSTRACT

Background: Parasites with complex life cycles depend on the ingestion of their intermediate host by the final host. To complete their life cycle successfully, parasites frequently manipulate the behaviour and appearance of the intermediate host. Within host–parasite systems, there is considerable variation in the intermediate host's behavioural response to infection.

Aim: Identify sources of parasite-induced variation in intermediate hosts' traits by focusing on intra- and inter-individual variation in behavioural responses to parasitic manipulation, taking infection intensity – and thus parasitic competition – into account.

Organism: The acanthocephalan parasite *Polymorphus minutus*, which alters the phototactic behaviour and activity of its intermediate host, *Gammarus pulex*, thereby increasing the probability of being eaten by the final host.

Methods: We repeatedly examined the behaviour of individual *G. pulex* varying in intensity of infection with *P. minutus* from uninfected to multiple-infected. We analysed phototactic responses and activity.

Results and conclusions: Individual gammarids differed in phototactic behaviour and in activity patterns, with repeatability ranging from 20% to 50%. Infected gammarids showed greater between-individual variation in phototaxis but not activity than uninfected gammarids. All uninfected gammarids were photophobic, whereas the phototactic behaviour of infected gammarids ranged from photophobia to photophilia. On average, multiple-infected gammarids were similarly photophobic as uninfected ones. Single-infected gammarids were less photophobic than uninfected and multiple-infected conspecifics. This suggests that intra-specific parasitic competition affects the manipulative abilities of parasites. Both groups of infected gammarids were on average less active than uninfected ones, and this effect was mainly driven by some infected individuals. In conclusion, behavioural variation of gammarids was caused

Correspondence: T. Thünken, Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany. email: tthuenken@evolution.uni-bonn.de

both by individual differences in responses to manipulation/infection, and by the reduced manipulative capacities of parasites facing intra-specific competition.

Keywords: behavioural manipulation, *Gammarus pulex*, individual variation, *Polymorphus minutus*, repeatability, host–parasite co-evolution.

INTRODUCTION

Parasites with a complex life cycle mature in an intermediate host species, but reproduce sexually in a different, final host species (Schmid-Hempel, 2011). In order to achieve the host change, it is often necessary that the intermediate host is ingested by the parasite's final host, i.e. *trophic transmission* (Lafferty, 1999). This creates a strong selective pressure on the parasite to increase the probability that its intermediate host is eaten by the final host (Moore, 2002). While there are convincing examples that parasites manipulate the intermediate host's behaviour and appearance to successfully complete their life-cycle in some host–parasite systems (Moore, 1983; Poulin and Thomas, 1999; Poulin, 2010; Bakker *et al.*, 2017), there remains debate as to what extent parasite-related changes in host phenotype increases transmission and whether these changes are adaptive for the parasite (Cézilly *et al.*, 2010).

According to the *manipulation hypothesis*, parasites that are able to disturb or reverse the anti-predator behaviour or cryptic appearance of their intermediate host should benefit from increased predation of the intermediate host (Moore, 2002). However, the evolutionary arms-race between intermediate host and parasites need not necessarily be won always by the individual parasite. This argument is supported by the occurrence of population-dependent, differential manipulative abilities of parasites (Franceschi *et al.*, 2010a). Unfortunately, few studies have examined individual behavioural variability of the intermediate host. Instead, parasitic effects have most often been examined using average values of behavioural or morphological traits of infected and uninfected host individuals. Such approaches, however, neglect within- and between-individual variation of host responses (Cézilly *et al.*, 2013; Poulin, 2013). As selection requires phenotypic variation at the individual level, detailed knowledge about variance components and the factors maintaining variation are crucial for a comprehensive understanding of the evolution of complex host–parasite systems (Thomas *et al.*, 2011). Such variation in manipulative effects might depend on, for instance, parasitic virulence (Alizon *et al.*, 2013), the intensity of infection and inter- as well as intra-specific interactions between parasites (Mideo, 2009; Cézilly *et al.*, 2014), but also on host resistance (Mazzi and Bakker, 2003; Daoust *et al.*, 2015).

Acanthocephalans represent a well-described example of manipulative parasites infecting arthropods as intermediate hosts and vertebrates as final hosts (Kennedy, 2006; Bakker *et al.*, 2017). Infection with an acanthocephalan leads to alterations in the appearance, behaviour, physiology, and life history of their intermediate hosts (for a review, see Bakker *et al.*, 2017). Some of these changes are caused by active parasitic manipulation while others are adaptive host responses to resist infection (Cézilly *et al.*, 2010; Bakker *et al.*, 2017). For example, the acanthocephalan *Pomphorhynchus laevis* uses various *Gammarus* species as intermediate hosts and certain fishes as final hosts (Kennedy, 2006). It alters the cryptic appearance of the intermediate host as the conspicuous orange cystacanth (the infective developmental stage of the parasite) is highly visible through the cuticle of the gammarid (Kennedy *et al.*, 1978). Such conspicuous colour makes the intermediate host more prone to predation by the threespine stickleback, *Gasterosteus aculeatus*, a suitable final host for *P. laevis* (Bakker *et al.*,

1997), but not to predation by *Salmo trutta*, an unsuitable host for *P. laevis* (Kaldonski *et al.*, 2009). Furthermore, the parasite does not only change the intermediate host's visual appearance, but also its anti-predator behaviour. While uninfected *Gammarus pulex* show predator avoidance and are photophobic, individuals infected with *P. laevis* are attracted by predator odour (Baldauf *et al.*, 2007) and show photophilic behaviour (Bakker *et al.*, 1997). These behavioural alterations are assumed to increase the probability of predation of the intermediate host, and thus the transmission of the parasite to the final host (Lagrue *et al.*, 2007). The acanthocephalan *Polymorphus minutus* exploits gammarids as intermediate hosts and water birds as final hosts (Kennedy, 2006). *Polymorphus* species alter the photo- and geotactic behaviour of the intermediate host, with infected amphipods being more photophilic and swimming closer to the water surface (Hindsbo, 1972; Bethel and Holmes, 1974; Bailly *et al.*, 2018). Furthermore, they reduce the overall activity of the intermediate host (Thünken *et al.*, 2010).

While such parasite-induced changes are well described at a mean population level, individual acanthocephalan-infected amphipods show considerable variation in behaviour (Thomas *et al.*, 2011), which can partly be ascribed to differential parasitic effects. For example, modification of the intermediate host's behaviour depends on the developmental stage of the parasite. *Pomphorhynchus laevis* and *Polymorphus minutus* are only infective at the cystacanth stage, not at the earlier acanthella stage [*P. laevis* (Franceschi *et al.*, 2008, 2010b), *P. minutus* (Bailly *et al.*, 2018)]. Consequently, parasites at different developmental stages have different interests, which are reflected in their manipulative potential (Dianne *et al.*, 2010, 2011). While the aim of individuals that have already reached the infective cystacanth is to increase predation of the intermediate host by the final host, that of younger individuals in the acanthella stage is to avoid predation (Hafer and Milinski, 2015). In addition, there are age-independent sources of manipulative variation. These include season-dependent effects (Benesh *et al.*, 2009; Franceschi *et al.*, 2010b; Bailly *et al.*, 2018), as well as genetic differences in the ability of individual parasites to manipulate the intermediate host (Franceschi *et al.*, 2010a). Furthermore, the parasitization intensity, i.e. the number of parasites within a single host, affects parasitic manipulation (Cézilly *et al.*, 2014). In multiple-infected hosts, cumulative parasitic effects might result in increased manipulation (Franceschi *et al.*, 2008). In contrast, competition between individual parasites over limited host resources might impede parasitic growth and development (Cornet, 2011; Dianne *et al.*, 2012), resulting in reduced manipulation (Caddigan *et al.*, 2017), especially when manipulation itself is costly (Maure *et al.*, 2013). Finally, parasites at different stages of their life cycle might have opposing interests, leading parasitic effects to cancel each other out, i.e. the *sabotage hypothesis* (Haine *et al.*, 2005; Dianne *et al.*, 2010; Hafer and Milinski, 2015).

Furthermore, differential responses to attempted manipulation by the parasite might be caused by variation of the host individual itself. This variation might be due to different responses between host individuals or high within-individual behavioural inconsistency. Infection may increase variation between hosts, for example, when certain individuals are susceptible to infection while others are more resistant. Furthermore, infected individuals may be less capable of maintaining consistency in behaviour, leading to higher within-individual variation compared with uninfected individuals.

To date, these different sources of variation in intermediate host responses have received limited attention, despite their importance for a complete understanding of parasite–host co-evolution. In the present study, we (1) describe within- and between-individual behavioural variation in infected and uninfected *G. pulex*, and (2) compare the intensity of

parasitic infection to changes in host behaviour. Therefore, we repeatedly tested photophobia and activity in individual gammarids over a period of 17 days. Test animals were either uninfected or carried at least one cystacanth of the manipulative parasite, *P. minutus*. To determine whether intra-specific competition within a host affects parasitic manipulation, single-infected (no competition for the parasite) or multiple-infected (competition between parasites) *G. pulex* were examined. The competition hypothesis as well as the sabotage hypothesis predict weaker manipulation of *Gammarus*. Alternatively, parasitic effects could accumulate, and thus multiple-infected hosts should suffer more as a result of manipulation.

MATERIALS AND METHODS

Experimental subjects

Uninfected, single-infected, and multiple-infected *Gammarus pulex* were collected on 10 May 2017 from a brook ('Derlebach') in Bonn, Germany (50°42′N, 7°02′E). At the capture site, the brook was approximately 50 wide × 15 cm deep. The water temperature was 10°C. Several hundred *G. pulex* were indiscriminately caught using a dip net and pre-sorted into infected and uninfected individuals. Gammarids were transferred to the laboratory using buckets filled with water and decaying leaves taken from the natural habitat. In the laboratory, the infection status of the gammarids was determined visually by checking for the presence and number of the orange cystacanths that were visible through the cuticle of the dorsal coelom (Bakker *et al.*, 1997). Furthermore, gammarids were measured and dissected directly after the experiments. Total length was defined as the distance between the base of the first antenna and the base of the telson, measured to the nearest millimetre with the animal placed on graph paper. Infected and uninfected gammarids did not differ significantly in size (uninfected: 10.38 ± 1.89 mm; single-infected: 10.07 ± 1.32 mm; multiple-infected: 9.38 ± 1.26 mm; mean ± SD; ANOVA: $F = 1.478$, $df = 2$, $P = 0.242$). After the experiment (see below), cystacanths were prepared from all infected individuals. They were photographed with tenfold magnification using a camera (Hitachi Denshi, HV-C20AMP) attached to a stereo-microscope (Leica, S8AP0). Photos were used to verify parasite species and infection status, i.e. number of parasites and developmental stage. All parasites were cystacanths of *P. minutus*. The parasites, ovoid in shape, were encased by an envelope and their proboscis was completely invaginated (Dezfuli *et al.*, 2001). The number of parasites in multiple-infected *G. pulex* varied between two and five (2.62 ± 0.26; mean ± SD).

In total, 13 uninfected, 13 single-infected, and 13 multiple-infected individuals were separated and kept individually in plastic boxes (18.5 cm long × 11.5 cm wide × 13.5 cm high) filled with 800 mL of aged tap water. Each box was equipped with an air stone and 2 g of decaying leaves, which served as food and shelter. Thus, individuals could choose between bright (open area) and dark (under the leaves) light conditions. About 70% of the water in each box was replaced once a week with aged tap water. A full-spectrum fluorescent tube (True-Light, Natural Daylight 5500, 36 W), emitting a spectral emission similar to natural daylight, was placed at a distance of 41 cm above the holding boxes, creating a maximum light intensity of 600 lux (PCE 174 Data logger light meter, PCE Instruments). Gammarids were kept at a 12 hour light/12 hour dark cycle and a temperature of 13 ± 1°C.

Experimental design

Experiments were conducted between 11 and 27 May 2017. Trials were performed on three consecutive days (Tuesday to Thursday) each week, with all individuals being tested once a day. Thus, each of the 39 gammarids was tested nine times. For the experiments, two clear plastic tanks, each measuring 24.5 cm long × 15 cm wide × 15.5 cm high, were placed on a white Styrofoam plate, with the longer sides aligned with each other (Fig. 1). Therefore, two trials could be conducted simultaneously. Tanks were filled with aged tap water to a level of 7 cm. The water temperature of the experimental tanks resembled holding conditions. The long sides of both tanks were covered with grey plastic sheets, so that light could only reach the tank from the short end and from above. The set-up was illuminated by a full-spectrum fluorescent tube (True-Light, Natural Daylight 5500, 36 W), installed at a height of 35 cm above the water surface and at a distance of 132 cm from one short side of the set-up. Thus, we created a brightness gradient within each tank (Fig. 1). The light intensity in the centre of the light-facing half of the respective tank was 39 lux; that in the centre of the half turned away from the light source was 31 lux. Above each tank we installed a webcam (Logitech, Webcam Pro 9000) connected to a laptop (Fujitsu Siemens, Lifebook SH531). For each trial, one gammarid was placed within a transparent plastic cylinder (diameter 3 cm) in the middle of each tank. After an acclimation phase of one minute, the cylinders in both tanks were lifted by hand, so that the gammarids were able to swim freely in their tank. Immediately after lifting the cylinders, video recordings were begun. A trial lasted 10 minutes. At the end of each trial, gammarids were carefully transferred back to their

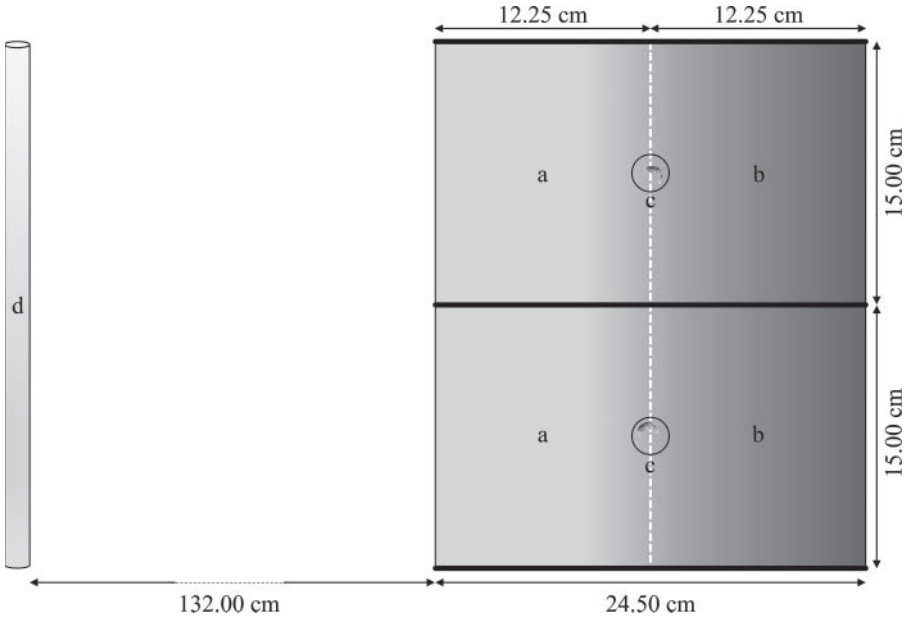

Fig. 1. Schematic representation of the experimental set-up. Two plastic tanks were placed alongside one another and separated visually. A brightness gradient was created by placing a slightly elevated light source (d) 132 cm away from one side of the set-up. For tracking-software analyses, two virtual zones were created with one facing the light source (a) and one facing away (b). The transparent cylinder (c) was lifted after one minute of acclimation time.

respective holding boxes. To exclude potential side-effects, the direction of the light source was switched after every fifth trial.

Motion analyses

Video recordings were analysed using the tracking software Biobserve Viewer III v.3.0.0.119 (Biobserve GmbH). The test tank was virtually divided into two equal-sized zones, one facing the light source (light) and the other one the opposite side (dark). The gammarid was tracked continuously throughout the 10 minute experimental phase. Time spent in each zone and changes between zones were determined and exported to Microsoft Excel. A phototaxis index was calculated (time on light side – time on dark side). Activity was estimated by the number of changes between the light and dark side.

Statistical analyses

Statistical analyses were conducted in R v.3.42 (R Development Core Team, 2013). When data deviated from normality, they were Box-Cox-transformed or non-parametric tests were applied. Between-individual differences in phototaxis and activity across and within infection groups (uninfected, single-infected, and multiple-infected) were examined by fitting linear models (lm) with individual gammarid as the explanatory variable. To test for between-individual behavioural variation among infections groups, we first calculated mean values for each gammarid and then used Levene tests to compare variation among infection groups. To compare within-individual variation, we first calculated a coefficient of variation (the ratio of the standard deviation to the mean) for each gammarid and then used Kruskal-Wallis rank sum tests to compare infection groups. Repeatability was calculated with the R package 'rptR' (see Stoffel *et al.*, 2017). To examine behavioural differences among infection groups, we applied linear mixed effect models (lme using the R package 'nlme') with activity or phototaxis as the dependent variable, infection group as the explanatory factor, and individual gammarid as a random factor. We added experimental day as a covariate to the model to examine whether phototaxis or activity changed over the course of the experiment and whether this relationship differed between infection groups (day × infection group interaction). Within infected gammarids, we investigated the effect of intensity of parasitization (number of parasites within a host) on phototaxis and activity, respectively, by fitting linear models. The relationship between phototaxis and activity was examined using a linear model with phototaxis (based on mean value, see above) as the response variable and activity as the explanatory variable. To test for differences in the relationship between phototaxis and activity between infection groups, we included activity × infection group as an interaction term in the model. All non-significant interaction terms were removed from the models (Engqvist, 2005). All tests were two-tailed, and alpha values less than 0.05 were considered to be statistically significant.

RESULTS

Phototaxis

Individual gammarids varied in phototaxis across infection groups (lm: $\Delta df = 38$, $F = 8.498$, $P < 0.001$; Fig. 2a) as well as within groups (lm: uninfected $\Delta df = 12$, $F = 2.973$, $P = 0.001$; single-infected $\Delta df = 12$, $F = 6.157$, $P < 0.001$; multiple-infected $\Delta df = 12$, $F = 8.503$,

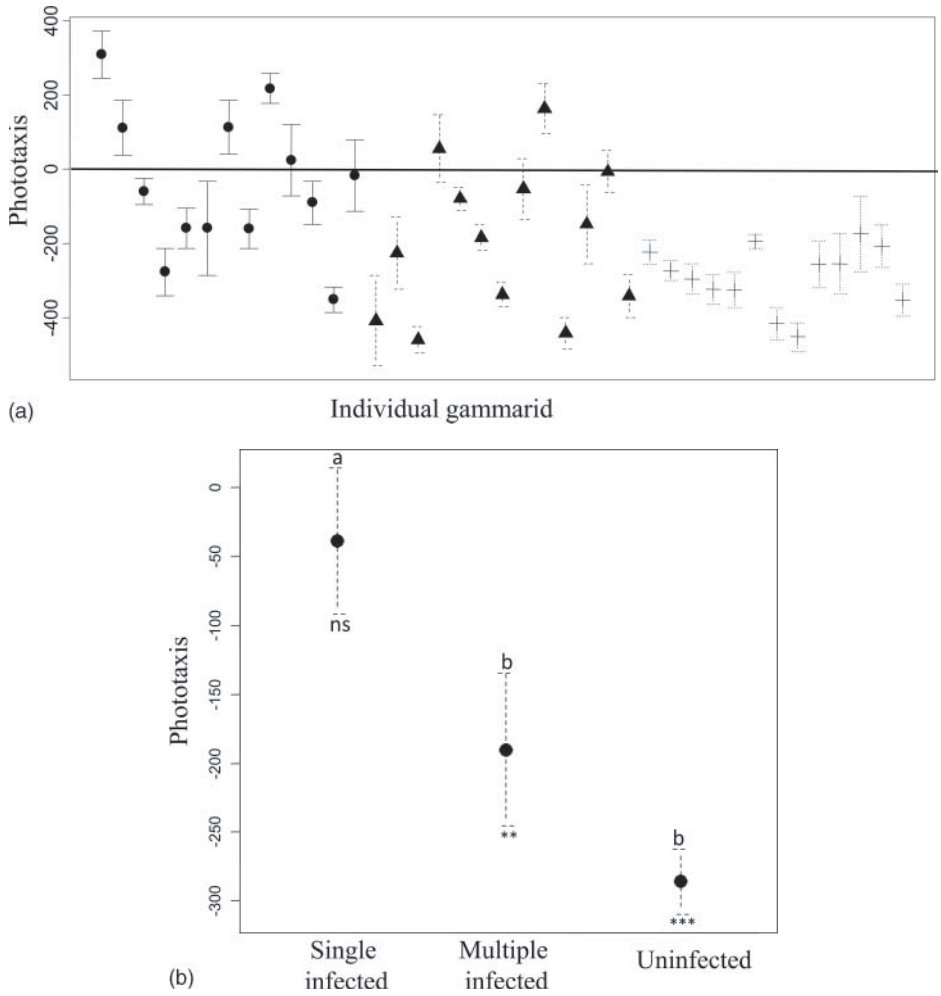

Fig. 2. (a) Phototaxis scores (time on light side minus time on dark side; values > 0 photophilic, values < 0 photophobic) for individual gammarids: single-infected (circles), multiple-infected (≥ 2 parasites, triangles), uninfected (crosses). Shown are mean values ± SE for each individual tested. (b) Mean (± SE) phototaxis values for the infection groups. Different letters above means indicate significant difference between groups ($P < 0.05$). Symbols below means indicate significant deviation from 0 (ns = $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$).

$P < 0.001$). Infection status influenced phototaxis (Table 1). On average, single-infected gammarids behaved randomly concerning phototaxis (one sample t-test: $t = -0.723$, $\Delta df = 12$, $P = 0.483$; Fig. 2b) and differed in phototaxis from both multiple-infected and uninfected gammarids (lme: both $\Delta df = 1$, both $\chi^2 > 3.897$, both $P < 0.05$; Fig. 2b). Multiple-infected and uninfected gammarids did not differ significantly from one another (lme: $\Delta df = 1$, $\chi^2 = 2.744$, $P = 0.100$; Fig. 2b) and both groups were on average photophobic (one sample t-tests: both $\Delta df = 12$, both $t < -3.420$, both $P < 0.01$; Fig. 2b). There were differences in individual variation in phototaxis between infection groups (Levene test: $\Delta df = 2$, $F = 4.142$, $P = 0.024$; Fig. 2a), with single- and multiple-infected gammarids being

Table 1. Results of linear mixed effect models (with individual as a random factor): effects of infection (uninfected, single-infected, and multiple-infected) and experimental day (day) on the phototaxis and activity of gammarids

Dependent variable	Interaction/fixed factor	N	Δdf	χ^2	P
Phototaxis	Infection group	39	2	11.732	**0.002**
	Infection group × Days	39	2	0.712	0.700
	Days	39	1	3.058	0.080
Activity	Infection group	39	1	7.609	**0.022**
	Infection group × Days	39	2	3.958	0.138
	Days	39	1	6.721	**0.009**

Table 2. Repeatability (R) with standard error (SE), 95% confidence intervals (CI), and P-values for phototaxis and activity for each infection group

Variable	Infection group	R	SE	CI	P
Phototaxis	Uninfected	0.192	0.098	0.006, 0.401	**0.003**
	Single-infected	0.382	0.118	0.127, 0.593	**<0.001**
	Multiple-infected	0.460	0.122	0.185, 0.647	**<0.001**
Activity	Uninfected	0.198	0.101	0.020, 0.406	**0.002**
	Single-infected	0.528	0.121	0.238, 0.710	**<0.001**
	Multiple-infected	0.496	0.126	0.195, 0.694	**<0.001**

more variable than uninfected ones (Levene tests: both $\Delta df = 1$, both $F > 5.800$, both $P < 0.025$; Fig. 2a). Single- and multiple-infected gammarids did not differ significantly in this respect (Levene test: $\Delta df = 1$, $F = 0.119$, $P = 0.732$; Fig. 2a). All uninfected gammarids avoided the illuminated side, whereas in infected gammarids we observed both photophobic and photophilic individuals, as well some that were irregular in their light response. During the course of the experiment (17 days), phototaxis did not change significantly (Table 1). Within-individual variation did not differ significantly between infection groups (Kruskal-Wallis rank sum test: $\Delta df = 2$, $\chi^2 = 0.560$, $P = 0.755$; see also Table 2 for repeatability values). Within infected gammarids, the number of parasites did not have a significant effect on phototaxis (lme: $\Delta df = 1$, $\chi^2 = 0.020$, $P = 0.886$).

Activity

Individual gammarids varied in activity across infection groups (lm: $\Delta df = 38$, $F = 8.467$, $P < 0.001$; Fig. 3a) as well as within groups (lm: uninfected $\Delta df = 12$, $F = 3.072$, $P = 0.001$; single-infected $\Delta df = 12$, $F = 10.886$, $P < 0.001$; multiple-infected $\Delta df = 12$, $F = 9.612$, $P < 0.001$). Infection status had an effect on activity (Table 1). On average, infected gammarids (single- and multiple-infected individuals) did not differ significantly from one another (lme: $\Delta df = 2$, $\chi^2 = 0.276$, $P = 0.599$), and were less active than uninfected individuals (lme: both $\Delta df = 2$, both $\chi^2 > 5.517$, both $P < 0.02$; Fig. 3b). Between-individual

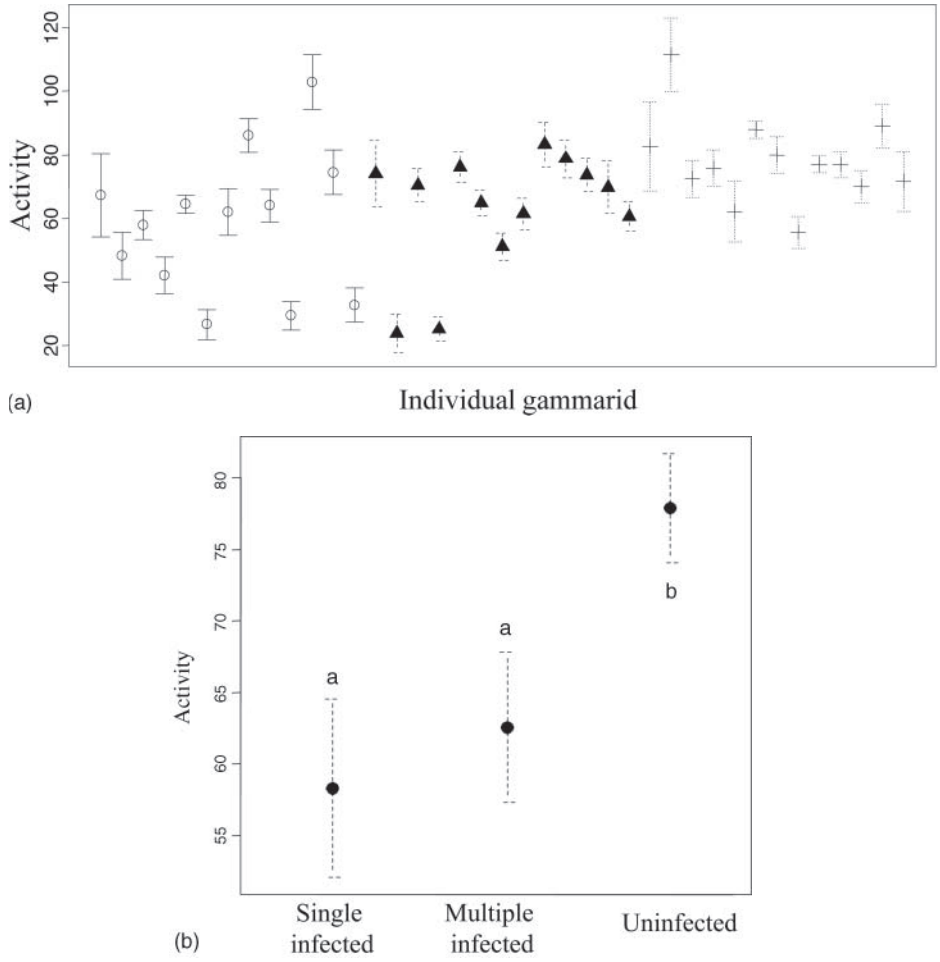

Fig. 3. (a) Activity, i.e. zone changes, for individual gammarids: single-infected (circles), multiple-infected (≥ 2 parasites, triangles), uninfected (crosses). Shown are mean values ± SE for each individual tested. (b) Mean (± SE) activity values for the infection groups. Different letters above means indicate significant difference between groups ($P < 0.05$).

variation was not significantly different between infected and uninfected gammarids (Levene test: $\Delta df = 2$, $F = 1.124$, $P = 0.336$; Fig. 3a). Individual coefficients of variation did not differ significantly between infection groups (Kruskal-Wallis rank sum test: $\Delta df = 2$, $\chi^2 = 0.560$, $P = 0.755$; see also Table 2 for repeatability values). In infected gammarids, the number of parasites did not have a significant effect on the host's activity (lme: $\Delta df = 1$, $\chi^2 = 1.854$, $P = 0.173$).

Relationship between phototaxis and activity

Phototaxis was not significantly correlated with activity (lm: $\Delta df = 1$, $F < 0.001$, $P = 0.984$). This effect was similar in infection groups (lm: activity × infection group interaction $\Delta df = 1$, $F = 0.361$, $P = 0.699$).

DISCUSSION

Understanding individual variation in behaviour is a classical topic in evolutionary and behavioural ecology research (Bakker, 1986; Bell *et al.*, 2009), and has recently been at the forefront of research within the framework of animal personality (Barber and Dingemanse, 2010; Beekmann and Jordan, 2017). In contrast, there has been much less interest in individual variation in host–parasite interactions (but see Thomas *et al.*, 2011; Poulin, 2013).

In the present study, both uninfected and *P. minutus*-infected gammarids showed repeatable individual differences in phototaxis and activity. Repeatability ranged from ~20% to 50%, values that are similar to those reported for other behavioural traits in a range of animal taxa (Bell *et al.*, 2009). Repeatability values for infected gammarids were higher than those of uninfected ones. This probably resulted from higher between-individual variation in infected gammarids compared with uninfected ones, as indicated by similar coefficients of variation between infection groups. In line with these findings, Benesh *et al.* (2008) found repeatable activity in isopods infected with *Acanthocephalus lucii*, but not in uninfected ones. In contrast, Coats *et al.* (2010) reported higher repeatability in uninfected amphipods compared with infected conspecifics. These contrasting results may reflect differences among species in manipulative capabilities of parasites or host resistances (Franceschi *et al.*, 2010a; Thomas *et al.*, 2011; see below).

Phototaxis

Between-individual variation in phototaxis was higher between infected individuals. While uninfected gammarids were uniformly photophobic (indicating strong selection on photophobia), infected individuals showed the full range of behaviour, from photophobia to photophilia.

The high variation observed in single-infected individuals might be explained by some cystacanths having not yet reached the manipulative stage. Indeed, even at the cystacanth stage further maturation or establishment within the host is required for manipulation to become apparent (Bethel and Holmes, 1974; Dianne *et al.*, 2010). Consequently, young cystacanths of *P. minutus* and *P. laevis* are less manipulative than older ones (Franceschi *et al.*, 2008; Bailly *et al.*, 2018). Bethel and Holmes (1974) showed that cystacanths of the closely related *Polymorphus paradoxus* induce alterations in the host just 17 days after reaching that stage. As we used naturally infected gammarids, we do not have information about the exact age of the parasite. However, if the described variation was caused by age effects, one would expect photophilia to increase over the course of the experiment in infected gammarids, as cystacanths aged during this time as well. As we did not find any significant time effects, the marked variation in manipulation most likely did not result from age differences between cystacanths. Rather, it might depend on the host's ability to resist manipulation, on individual parasites' manipulative abilities, or a combination of the two. Indeed, it has been shown that sibships of the manipulative acanthocephalan *P. laevis* differ in manipulative ability (Cornet *et al.*, 2009; Dianne *et al.*, 2012) and that gammarid hosts can develop resistance against local manipulative parasites (Franceschi *et al.*, 2010a).

Interestingly, mean photophobic responses of multiple-infected gammarids were comparable to those of their uninfected conspecifics. Thus, a multiple infection did not lead to a stronger response. In contrast, our results suggest that intra-specific competition among parasites dampens their manipulative effects. This effect can be explained in two

ways. First, intra-specific competition within the host might have affected parasites' development (Dezfuli *et al.*, 2001; Franceschi *et al.*, 2008; Dianne *et al.*, 2010). Provided that host resources are limited, and manipulation is costly, cystacanths sharing a host may need longer to reach maximum manipulative potential. Second, parasites at different developmental stages will have different manipulative interests. While the aim of older, highly infective cystacanths is to be predated by a bird, younger ones should favour remaining in the intermediate host for longer. Thus, lower photophilic behaviour might be explained by cystacanths actively competing for control of their *Gammarus* host.

Activity

In line with previous studies (e.g. Thünken *et al.*, 2010), infected gammarids were less active compared with their uninfected conspecifics. Interestingly, multiple-infected individuals showed similar activity to single-infected gammarids and the number of parasites within a gammarid was not significantly correlated with activity. This suggests that the additional load afforded by the parasite is not responsible for the changes in the host's activity. Although infected gammarids were on average less active, a proportion of infected individuals displayed similar activity to uninfected ones (cf. Fig. 3a), suggesting that specific individuals only show changes in activity or that reduced activity is only present at a specific time point, e.g. when the parasite actively interferes with the physiology of the host. Furthermore, in contrast to phototaxis, variation in activity among individuals was similar between infection groups, supporting the findings of previous studies that changes in activity are side-effects of the infection rather than the result of active manipulation (e.g. Poulin, 1994; Thünken *et al.*, 2010). Future research should address these questions in more detail.

Another source of individual variation within infection groups might be the sex of the gammarids. Indeed, acanthocephalan parasites reduce female fecundity (Bollache *et al.*, 2002). However, evidence for sex-specific behavioural responses to infection is ambiguous. Park and Sparkes (2017) found that *Acanthocephalus dirus*-infected males and females of *Caecidotea intermedius* differ in refuge use, while Bailly *et al.* (2018) did not find sex-specific phototactic responses of *P. minutus*-infected gammarids. We did not explicitly determine the sex of the gammarids used in our study. However, animals of the different infection groups were similar in size. Given the size-range of the animals used, suggests that we used both male and female *G. pulex* (Adams and Greenwood, 1983). Therefore, the differences between the three different groups cannot be explained by sex differences. However, the variability within the infected groups might be caused by different reactions of infected males and females. This hypothesis might be investigated in more detail in future studies.

In summary, we have shown that individual gammarids differ in their risk-adverse behaviour. Furthermore, we found high variation in manipulative success of an acanthocephalan parasite, which could be explained by between-parasite competition within an intermediate host and differential responses of individual hosts to manipulation. Such variation in responsiveness underlines the ongoing arms-race between the parasite and its host and sheds light on the evolution of trophic-transmitted parasites and their hosts.

AUTHOR CONTRIBUTIONS

T.T., S.V., and S.A.B. conceived the study. S.V. and T.J. conducted the experiments. T.T. analysed the data. T.T., S.V., and J.G.F. discussed the results and wrote the manuscript. All of the authors approved the final draft of the manuscript.

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