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Effects of parasites upon non-host predator avoidance behaviour in native and invasive gammarids

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Research Article

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Abstract

The acanthocephalan parasite, *Polymorphus minutus*, manipulates its intermediate hosts' (gammarids) behaviour, presumably to facilitate its transmission to the definitive hosts. A fundamental question is whether this capability has evolved to target gammarids in general, or specifically sympatric gammarids. We assessed the responses to chemical cues from a non-host predator (the three-spined sticklebacks *Gasterosteus aculeatus*) in infected and non-infected gammarids; two native (*Gammarus pulex* and *Gammarus fossarum*), and one invasive (*Echinogammarus berilloni*) species, all sampled in the Paderborn Plateau (Germany). The level of predator avoidance was assessed by subjecting gammarids to choice experiments with the presence or absence of predator chemical cues. We did not detect any behavioural differences between uninfected and infected *G. pulex* and *E. berilloni*, but an elevated degree of predator avoidance in infected *G. fossarum*. Avoiding non-host predators may ultimately increase the probability of *P. minutus*' of predation by the definitive host. Our results suggested that *P. minutus*' ability to alter the host's behaviour may have evolved to specifically target sympatric gammarid host species. Uninfected gammarids did not appear to avoid the non-host predator chemical cues. Overall the results also opened the possibility that parasites may play a critical role in the success or failure of invasive species.

Introduction

The 'increased host ability hypothesis' posits that parasites manipulate the behaviour of their intermediate hosts in order to avoid predation by non-host species in order to enhance their transmission to the next host (Médoc and Beisel, 2008). The life cycle of acanthocephalan parasites includes intermediate hosts, such as amphipods, isopods, ostracods, copepods, insects and myriapods (Nickol, 1985). The acanthocephalan parasite, *Polymorphus minutus*, appears capable of altering the behaviour of its intermediate hosts (amphipods) in a manner that enhances their transmission to the definitive host, the water fowl (Bakker *et al.*, 1997; Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007; Médoc and Beisel, 2008).

Earlier studies have demonstrated that parasites decrease non-host predator exposure by manipulating their host's predator avoidance behaviour (Holomuzki *et al.*, 1988; Rigby and Jokela, 2000; Matz and Kjelleberg, 2005; Friman *et al.*, 2009). Parasites modify the host's behaviour by regulating the host's neurotransmitter serotonin levels (Tain *et al.*, 2006; Cézilly and Perrot-Minnot, 2010; Cézilly *et al.*, 2013) or through the production of anaerobic metabolites (such as lactate and succinate; Perrot-Minnot *et al.*, 2016). However, one fundamental question remains; has such ability to manipulate the host's behaviour evolved specifically to target the host genus in general, or only sympatric, co-existing host species.

The aim of this study was to assess if modulation of the host's behaviour by *P. minutus* is specific to sympatric gammarids or gammarids in general. Earlier studies suggested that modulation of the host behaviour evolved through 'adaptation' (Baldauf *et al.*, 2007) and 'exaptation' (Combes, 2005; Beisel and Médoc, 2010) by parasites or both (Cézilly and Perrot-Minnot, 2005). Exaptation describes the case when a behavioural trait (e.g. negative geotaxis) in *P. minutus*, that evolved in response to predation, ultimately facilitates trophic transmission to the definitive host, i.e. due to an elevated level of avoidance towards non-host predators (Rohde, 1994; Beisel and Médoc, 2010).

The native gammarids, *Gammarus pulex*, and, *Gammarus fossarum*, are abundant in Central European upper and middle freshwater tributaries from Danube to Rhine drainage systems (Siegismund and Müller, 1991). In contrast, the gammarid *Echinogammarus berilloni* is native to the Atlantic regions of France and Spain (Médoc *et al.*, 2015), but is an invasive species in western and Central Europe (Schmidt-Drewello *et al.*, 2016). The gammarids are predated upon by water fowl, which are *P. minutus*' definitive hosts, but also by non-host predators, such as three-spined sticklebacks, *Gasterosteus aculeatus* (Médoc *et al.*, 2009). The three-spined sticklebacks are native to the Paderborn Plateau in Westphalia, Germany (Schmidt-Drewello *et al.*, 2016). Consequently, avoiding predation of gammarid hosts by non-

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host predators would increase the probability of predation of gammarids by water fowl and hence their transmission to the definitive host.

The objective of this study was to assess if *P. minutus* infected gammarids altered the gammarids behaviour towards non-host predators, more specifically three-spined sticklebacks, and if such a change was observed in all, or only sympatric, gammarid species. We tested whether the 'increased host ability hypothesis' in non-host predators, was more pronounced in native, congenerics, sympatric co-evolved gammarids in comparison to sympatric invasive gammarids.

Materials and methods

Sampling

Specimens of *G. pulex* were collected in the Altenau river (51° 35′56.73″N;8°46′56.92″E), whereas specimens of *G. fossarum* and *E. berilloni* were collected in the upper and lower parts of the Alme river, respectively (51°32′50.05″N; 8°32′43.61″E). Sampling took place during May and June in 2010. The prevalence of *P. minutus* in all three gammarids was below 1% in the Paderborn Plateau river system (Niehof and S. Farahani, unpublished data). Both river systems are also home to several fish species that prey on gammarids, such as three-spined sticklebacks (Schmidt-Drewello *et al.*, 2016), river trout *Salmo trutta fario*, minnow *Phoxinus phoxinus*, northern pike *Esox Lucius*, chub *Squalius cephalus*, rainbow trout *Oncorhynchus mykiss*, freshwater sculpin *Cottus gobio* and grayling *Thymallus thymallus*. Both tributaries are located on the Paderborn Plateau in Westphalia, Germany. All sampled gammarids were kept in Dewar flasks in water from the sampling site during transport (~90 min). On arrival in the laboratory sampled gammarids were transferred to aquaria (50 × 50 × 25 cm) with aerated tap water at 16 ± 1°C (water temperature at sampling site) and kept for two or three weeks. Infected and uninfected gammarids were kept in separate aquaria. Infected gammarids were identified from the number of orange-red spots (cystacanth, the developmental stage at which the parasite is able to infect its definitive hosts) present on the cuticle (Dezfuli and Giari, 1999). A dark:light cycle of 10:14 h was maintained in order to mimic seasonal conditions. Gammarids were fed beech leaves, *Fagus sylvatica*. Feeding was ceased 48-h period prior to experimentation in order to induce foraging behaviour. The three-spined sticklebacks employed during experimentation were F1 offspring of wild-caught individuals and were kept in an aerated aquarium (62 × 81 × 30 cm). In order to reduce potential confounding effects from the presence of fish faeces and residual food, the feeding (with chironomid larvae) of the three-spined sticklebacks was ceased 72 h prior to experimentation. The water in all aquaria was filtered and dechlorinated tap water (pH 7.4; EC 0.5 mS cm^{-1}) was used.

Experimental setup

Laboratory experiments were conducted in a climate chamber at 16 ± 1°C water temperature during May, June and July in 2010. Gammarid non-host predator avoidance was assessed using a choice setup (Fig. 1) configured as a Y-shaped maze with a basal stem (5 × 5 × 15 cm) that split into two separate upper stems (5 × 5 × 10 cm). The floor of the maze was covered with 4–6 mm gravel. Tap water was circulated into the maze through the upper stems towards the main basal stem at a rate of *ca.*2.3 cm^3 s^{-1}. Preliminary tests with ink and salt, respectively, revealed that a flow rate of 2.3 cm^3 s^{-1} ensured mixing of two aquaria water in the basal stem, but prevented mixing of water in the two upper stems. The inflow water originated from one of two aquaria (each

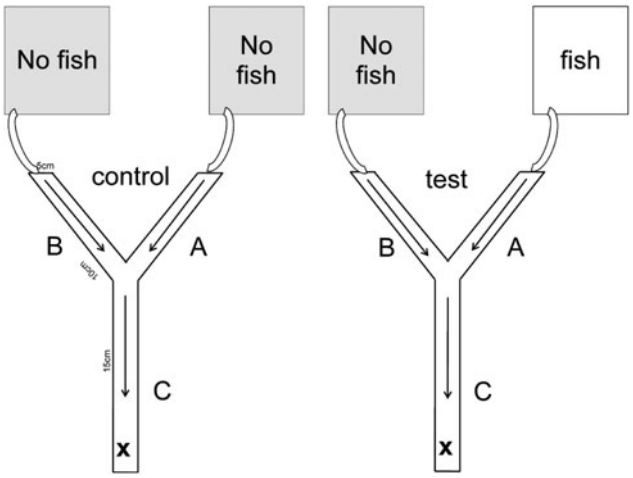


Fig. 1. Two-choice microcosm setup. A (control) = inflow from the aquarium without fish, A (test) = inflow from the aquarium with fish, B (control, test) = inflow from the aquarium without fish, C = mixing area, arrows = direction of flow, × = release point of gammarids.

32 × 17.5 × 19 cm). One aquarium contained tap water with 10 three-spined sticklebacks introduced into the aquarium 48 h before the initiation of the experiment. The other aquarium contained tap water only. During control experiments both aquaria contained only tap water. Non-host predator avoidance was assessed in uninfected and *P. minutus* infected gammarids. Experiments were categorized into either 'I' (*P. minutus* infected gammarids), or 'U' (uninfected gammarids). Control (i.e. no three-spined sticklebacks in either aquarium) and test (i.e. one aquarium without and the other with three-spined sticklebacks) experiments were denoted 'C' and 'T', respectively. For instance, the combination UT denoted an experiment with uninfected gammarids during which one aquarium contained only tap water and the other aquarium 10 three-spined sticklebacks. Individual gammarids were randomly chosen for each trial. The number of *G. pulex*, *G. fossarum* and *E. berilloni* are 82, 53 and 45 (UT), 32, 36 and 26 (IT), 30, 32 and 32 (UC), 32, 31 and 32 (IC), respectively. The water of the aquaria during the test and control experiments was aerated but oxygen levels within the aquaria were not tested. The length of the gammarids was measured using imageJ (ver. 1.35, https://imagej.nih.gov/ij/index.html) from the uropod to the pedunculus along the dorsal side. The number of cystacanths of each individual gammarid was measured. Gammarids shorter than <8 mm and with more than one cystacanth were excluded as recommended by Bauer *et al.* (2005) and Medoc and Beisel (2008) in order to avoid possible effects due to differences in size or degree of infection. Our experiment was designed to ensure 'fresh' fish chemical cues, which was presumed to alert the test specimens that a non-host predator was nearby.

Data collection

All experiments were conducted with single gammarids. The test specimen was released into the area at the base of the main stem (denoted area C, Fig. 1). Movement from area C to either areas A and B was restricted with a plastic barrier in area C for 300 s after which the test specimen could move freely within the maze for 300 s. The location of each specimen was recorded every 30th second. Specifically, the duration spent in areas C, A and B was noted (Fig. 1). The source of inflow water into areas A and B (i.e. aquaria with or without three-spined sticklebacks, respectively) was alternated between experiments. After the completion of an experiment, each specimen was re-checked for

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infection by other parasites. Gammarids infected with acanthocephalan parasitic worms *Pomphorhynchus* spp., were identified from the clearly visible orange-yellow parasites behind the transparent cuticle (Bakker *et al.*, 1997). Infection by *Echinorhynchus* spp., was identifiable as an orange-/red-coloured worm visible in the pereon or the pleon (Macneil *et al.*, 2003). Gammarid specimens identified as infected with parasite, other than *P. minutus*, or infected with *P. minutus* in the acanthella stage (non-infective to the definitive host) were excluded from the analysis. We did not identify other parasites in gammarids infected with *P. minutus*. We observed one *Echinorhynchus* spp. in an uninfected *P. minutus* individual. This individual was subsequently excluded from the analysis.

Statistical analyses

We modelled the transitions between areas A, B and C using a discrete-time Markov chain approach, i.e. an individual at location $Z_t = x$ at time t makes a transition to area $Z_{t+1} = y$ at time $t + 1$with probability

$$Pr(Z_{t+1} = y | Z_t = x) \equiv p_{x \to y} \tag{1}$$

In other words, we estimated transition matrices of the form,

$$M = \begin{bmatrix} p_{A\to A} & p_{B\to A} & p_{C\to A} \\ p_{A\to B} & p_{B\to B} & p_{C\to B} \\ p_{A\to C} & p_{B\to C} & p_{C\to C} \end{bmatrix} \tag{2}$$

All three columns of M sum to 1; hence, M represents six independent parameters. In the full model, M was allowed to vary simultaneously with the three predictor variables: time $t \in \{1\ldots 10\}$, species $s \in \{EB, GF, GP\}$ and treatment combination $T \in \{UC, IC, UT, IT\}$.

All specimens started from area C, and hence the first transition corresponds to two parameters ($p_{C\to A}$,$p_{C\to B}$,$p_{C\to C} = 1 - p_{C\to A} - p_{C\to B}$) whereas each of the subsequent nine transitions corresponded to six parameters. In total, the full model was therefore comprised of $(2 + 9 \times 6) \times 3 \times 4 = 672$ parameters. Simpler models were assessed by excluding one or more predictor variables.

Given the transition probabilities $p_{x\to y}$, the probability $Pr(Z_t = y)$ of presence in area y at time t was estimated recursively:

$$Pr(Z_t = y) = \sum_x Pr(Z_{t-1} = x) p_{x\to y} \tag{3}$$

Finally, given the above time-dependent probabilities, we estimated the average probability of presence at a given area as the following:

$$Pr(Z = y) = \frac{1}{10} \sum_{t=1}^{10} Pr(Z_t = y) \tag{4}$$

The transition probabilities in each column of M were estimated by treating the observed transition frequencies at a given area as drawn from a multinomial distribution. We fitted Bayesian Markov Chain Monte Carlo (MCMC) models with the brms interface (version 2.7.3; Bürkner, 2016) to RStan (Stan Development Team, 2018), using RStudio (version 1.2.1268; RStudio Team, 2018) with R (version 3.5.0; R Development Team, 2018). We used flat priors for the transition probabilities. Each estimation was run with four MCMC chains of 2500 iterations each, preceded by a burn-in period of 1000 iterations, thus yielding a total of 10 000 samples per parameter per model. Mixing and convergence of chains was assessed from the trace plots and the Gelman–Rubin r-hat statistic (Gelman *et al.*, 1992). In all cases r-hat was <1.001, which suggested convergence. The effective sample size for all parameter estimates was above 1000. The mean and the 89th percentile intervals (PI) of the marginal posterior densities were reported for all parameters.

Table 1. Comparison of Bayesian multinomial Markov chain models

Model	$\Delta WAIC$	Weight
Species×treatment	0.0	1.00
Treatment	92.4	0.00
Species	127.1	0.00
Time×treatment	282.7	0.00
Time×species	293.8	0.00
Time	301.9	0.00
Time×species×treatment	322.1	0.00

The models were ranked by their *WAIC* scores from low to high. $\Delta WAIC$ denotes the specific model's difference in *WAIC* score relatively to the model with the lowest *WAIC* model. Weight indicates the posterior model weight [equation (5)]

Different combinations of predictor variables were assessed using the Watanabe–Akaike Information Criterion (WAIC; Watanabe, 2010). For each model m among a set of models R, we calculated the model weight according to

$$\omega_m = \frac{\exp\left(-\frac{1}{2}\Delta WAIC_m\right)}{\sum_{m\in R} \exp\left(-\frac{1}{2}\Delta WAIC_m\right)} \tag{5}$$

The statistic $\Delta WAIC_m$ referred to the difference in WAIC value between model m and the best-fitting model (i.e. the model with the lowest WAIC score; this model itself has $\Delta WAIC = 0$). In total, seven models each with three predictor variables were compared. The models were: species×treatment (fish presence or absence), treatment, species (amphipod species), time×treatment, time×species, time×species×treatment and time.

Results

The 'species×treatment' model (Table 1) was the model with the highest support at a weight of *ca.*1.0 among the seven competing models. No evidence of time-dependent transition probabilities was observed, i.e. all four models including time as a predictor variable were ranked lowest. In general, all test specimens tended to move away from the initial area C during the experiment and approached quasi-equilibrium frequencies in the three areas A, B and C (Fig. 2).

The invasive species, *E. berilloni* showed no statistically significant preference for area A over B or vice versa in either of the four treatment categories (Fig. 3). In contrast the native species, *G. fossarum* and *G. pulex*, showed a significant preference, mostly for area A, in six out of eight treatment categories (Fig. 3). In five out of six comparisons, between control (no non-host predator present) and test (non-host predator present) experiments, presence in area C decreased significantly when the non-host predator was present. The presence of infected *G. fossarum* and *G. pulex* in area C was more frequent in the treatment group compared to uninfected individuals (Fig. 3).

The most pronounced effect of non-host predator presence was observed in both uninfected native gammarids, with an

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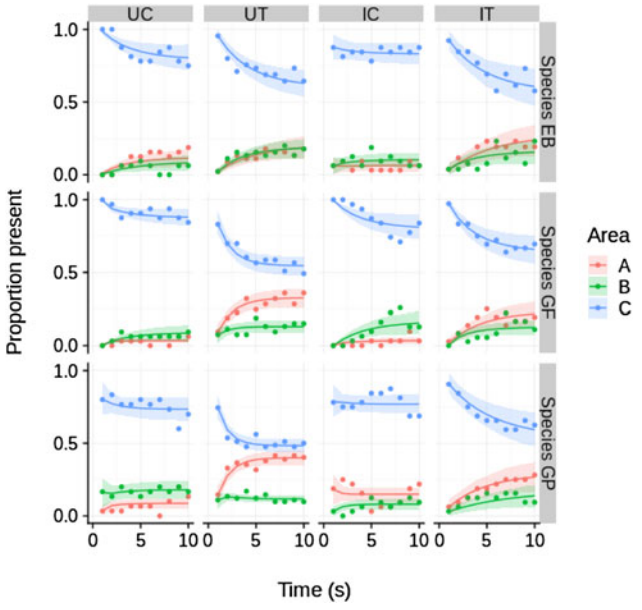


Fig. 2. Temporal dynamics of proportional presence (Y axis) in areas A, B and C of the experimental aquarium of *GP* (*G. pulex*), *GF* (*G. fossarum*), and *EB* (*E. berilloni*) for the four treatment combinations (UC, UT, IC, IT). Smoothed graphs (thick lines) are predictions of the best supported model (predictor species×treatment; see Table 1) while raw data are represented by dots. The shaded areas represent the 89% highest posterior density interval (HPDI) for the predicted values. Time (*X* axis) indicate 10 recordings (one per in 30 s). UC, uninfected control; UT, uninfected test; IC, infected control; IT, infected test.

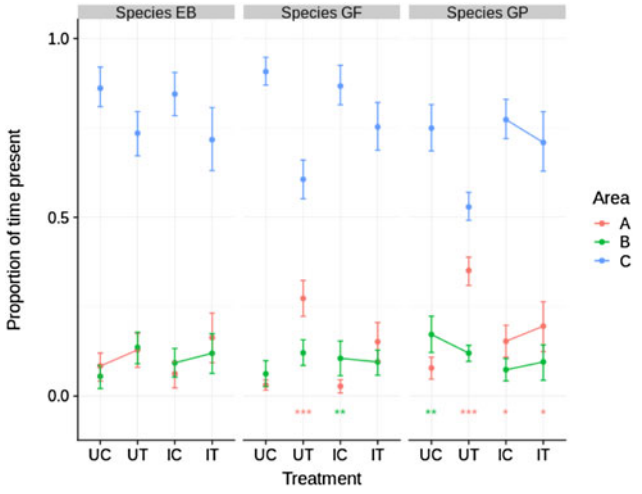


Fig. 3. Proportion of time present in different areas for all combinations of three species and four treatments. Dots indicate posterior means and error bars the 89% HPDI. Asterisks near the bottom indicate within-treatment significance of the difference between time spent at A and time spent at B, i.e. posterior probability of Pr(A) – Pr(B) >0 (red) or Pr(A) – Pr(B) <0 (green) (* $P > 0.95$, ** $P > 0.99$, *** $P > 0.999$). Lines between adjacent control and test treatments within areas indicate a non-significant difference ($P \geqslant 0.05$), whereas absence of lines indicates a significant difference ($P \leqslant 0.05$). UC, uninfected control; UT, uninfected test; IC, infected control; IT, infected test.

increase in preference for area A; the area closest to the non-host predator. Infected native gammarids also had a tendency towards increased presence in area A (statistically significant for *G. pulex*, but not in the case of *G. fossarum*), although this effect was significantly lower than was the case for uninfected gammarids (posterior probabilities of $Pr(A)_{UT} - Pr(A)_{UC} > Pr(A)_{IT} - Pr(A)_{IC}$ were 0.20, 0.99 and 1.00 for *E. berilloni*, *G. fossarum* and *G. pulex*, respectively).

Discussion

Avoidance behaviour of uninfected and infected gammarids to non-host predator

The estimated increase in avoidance behaviour towards the non-host predator chemical cues observed in *G. fossarum* infected with *P. minutus* in our study was consistent with the 'increased host ability hypothesis', which predicts that parasites increase the avoidance towards non-host predators (Medoc and Beisel, 2008). In Westphalia, the native cryptic parasite *P. minutus* uses native *G. fossarum* as an intermediate host but the *P. minutus* co-invaded the region with invasive gammarids, *E. berilloni* and *G. roeseli* from the Mediterranean area and Southeast Europe, respectively (Zittel *et al.*, 2018). *Gammarus roeseli* transmitted this invasive parasite to *G. pulex* (Zittel *et al.*, 2018). In contrast, native *G. pulex* infected with *P. minutus* displayed no non-host predator avoidance perhaps because invasive *P. minutus* have not evolved this mechanism in the presence of the native *G. pulex*. Perhaps native *P. minutus* can potentially have different traits than invasive *P. minutus* in manipulating the intermediate host, *G. fossarum*, behaviour in such a way that it is increasing the chances of being transmitted to the definitive host. The weak effects on non-host predator avoidance in infected gammarids detected in our study could be due to the fact that our experiments were conducted without the host predator chemical cues, making it more difficult for individuals to discern between different predator chemical cues and to behave accordingly. Our results would probably be different if we included both host predator and non-host predator in our experiment, e.g. Jacquin *et al.*, 2014. We agree that it is worthwhile to carefully contemplate when it is appropriate to make the inference regarding the specificity of gammarid behaviour in relation to 'non-host' predators *vs* predators in general. Since our experiments did not include a host predator we were unable to compare behavioural responses towards these two predator groups. Accordingly, we cannot state with any certainty, that the observed responses were specifically due to non-host predator presence, i.e. the behaviour could have been elicited because of predator presence *per se*.

The realization of the presence of the predator by the prey possibly occurs indirectly through the detection of pheromones and chemical cues from other prey species released during predation (Dicke and Grostal, 2001) or directly *via* chemical cues from the predator (Kats and Dill, 1998). In aquatic environments, the prey detects predators mostly *via* chemical cues (Dodson *et al.*, 1994; Chivers *et al.*, 1996; Hettyey *et al.*, 2010). For example, gammarids respond to chemical cues from predators at low visibility by reducing activity and move towards the bottom (Wisenden *et al.*, 1999).

This study also revealed that uninfected gammarids had a preference towards non-host predators. This behaviour was especially noticeable in the native gammarids compared to the invasive gammarid. However, these results did not agree fully with our expectations, as native gammarids preferentially moved towards non-host predator chemical cues whereas invasive gammarids reduced their activity in the presence of non-host predator chemical cues but did not appear to actively avoid non-host predator chemical cues. The results of this study were in agreement with those previously reported by Schmidt-Drewello *et al.* (2016), who found that uninfected invasive *E. berilloni* appeared more capable of detecting chemical cues from three-spined stickleback compared to uninfected native gammarids, *G. pulex* and *G. fossarum*. There may be several causes for the observed absence of predator avoidance towards non-host predators by uninfected native gammarids. Uninfected native gammarids appear able to discern between the chemical cues emitted by efficient predators [specialist predators with using efficient

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hunting behaviours and persisting to eat preferred prey items (Spencer *et al.*, 2017) e.g. sculpin, *Cottus gobio*, trout, *Salmo trutta*, in the Stampen Stream in southern Sweden (Abjornsson *et al.*, 2000), perch, *Perca fluviatilis*, Lake Lucerne in Switzerland (Baldauf *et al.*, 2007) and gudgeon, *Gobio gobio*, in Gauernitzbach near Dresden,Germany (Szokoli *et al.*, 2015)] and those chemical cues emitted by inefficient predators, i.e. scavengers or predatory species that are inefficient in targeting gammarids [e.g. cray fish, *Pacifastacus leniusculus*, in the Stampen Stream (Abjornsson *et al.*, 2000), stone loach, *Barbatula barbatula*, in Gauernitzbach (Szokoli *et al.*, 2015) and three-spined sticklebacks in Lunain river at Nonville, France (Jacquin *et al.*, 2014)]. Our results may suggest the underlying cause of the observation that uninfected, invasive gammarids limit their range to areas with fish predators relative to the range of uninfected native gammarids on the Paderborn Plateau (Keane and Crawley, 2002). However, this needs further investigation. Another cause for the observed absence of predator avoidance towards non-host predators by uninfected native *G. pulex* is its local adaptation in the presence of fish.

Gammarus pulex is an active and explorative amphipod (Truhlar and Aldridge, 2015). It lives in shallow muddy streams where low water depth and dense benthic detritus may reduce the risk of detection by fish (Haddaway *et al.*, 2014). *Gammarus fossarum* is abundant in the upper reaches of streams or in the mountainous areas, where the only predatory fishes are salmonids. Presumably, *G. fossarum* has not been exposed to a diverse array of fish predators (Siegismund and Müller, 1991; Müller, 2000; Pöckl *et al.*, 2003).

Fish chemical cues could signal the presence of potential food resources to gammarids, Gammarids are omnivore scavengers that feed on carcasses and fish faeces (Wilhelm and Schindler, 2011; Jermacz *et al.*, 2017). However, our results were not confounded by fish chemical cues. The three-spined sticklebacks in our experiment, were not fed 72 h prior to the experiments in order to remove such chemical cues (Wisenden *et al.*, 1999; Ward, 2012; Smith and Webster, 2015). In our experiment, the presence of fish faeces was not recorded. However, given the potential significance of fish faeces as food for amphipods, future studies should attempt to disentangle whether fish chemical cues and/or the presence of fish faecal matter influences gammarid behaviour.

Our experiment employed chemical cues from non-host predator that were present in the vicinity of the gammarid during the experiment, presumably alerting the individual gammarid of their proximity. Indeed, the presence of infected and uninfected gammarids in the release area (C) decreased (but not always) when 'fresh' chemical cues of non-host predator were present. Unwillingness to presence in the release area was a specific case and confirmed increasing gammarids activity after detecting the source of chemical cues and confirmed increasing the time duration of presence of infected and uninfected gammarids in area with non-host predator chemical cues. Our results were in contrast with previous studies that demonstrated a decrease in prey activity when predators were present, such as, reduction feeding activity [e.g. in tadpoles, *Rana temporaria* (Van Buskirk *et al.*, 2014)] and swimming, crawling and downstream drift [e.g. in *Hyalella azteca* (Stone and Moore, 2014), *Gammarus lacustris* (Wudkevich *et al.*, 1997) and *Echinogammarus stammeri* (Dezfuli *et al.*, 2003)].

Intra-specific comparison between uninfected and infected gammarids

No preference for areas with and without three-spined stickleback chemical cues was observed in either uninfected or infected *G. pulex*. This observation was in agreement with previous reports by Kaldonski *et al.* (2007), who did not detect any significant differences in the use of refuges between infected or uninfected *G. pulex* to chemical cues from freshwater sculpin, *Cottus gobio*, a non-host predator. However, Kaldonski *et al.* (2008), showed that water scorpions, *Nepa cinera*, predated upon uninfected *G. pulex* more frequently than infected *G. pulex*.

We found that infection by *P. minutus* altered the non-host predator avoidance in native *G. fossarum*, in a manner that decreased the probability of presence in the area with three-spined stickleback chemical cues, compared to uninfected *G. fossarum*. Conversely, neither infected nor uninfected *E. berilloni* showed a significant preference for a specific area (with or without three-spined stickleback chemical cues) in either of the four treatment categories. Jacquin *et al.* (2014) found that infected *E. berilloni* by *P. minutus* under the adaptationist hypothesis (Baldauf *et al.*, 2007) reduced their activity in the presence of fish chemical cues, although modulating the intermediate host's behaviour is not always adaptive for the parasite (Bakker *et al.*, 2017). However, Jacquin *et al.* (2014) employed native *E. berilloni* from the Lunain river (France) and non-host predator chemical cues from both three-spined stickleback and minnows, *Phoxinus phoxinus*. Our findings indicated that there was a difference in non-host predator avoidance behaviour of infected *E. berilloni* on the Paderborn Plateau in comparison to their native origin area in France.

The outcome of interactions between native and invasive hosts and their parasites are shaped by an array of complex ecological and evolutionary factors (Alexander *et al.*, 1996; Parker and Gilbert, 2004) affecting the success and intensity of invasions by non-native species (MacNeil *et al.*, 2003; Prenter *et al.*, 2004). The fate of invasive species is, in part, governed by competition between native and invasive species according to the 'evolution of increased competitive ability' hypothesis (Blossey and Nötzold, 1995) as well as competition between uninfected and infected hosts during the invasion according to the 'increased host ability hypothesis' in order to reach the parasite maximum fitness and enhance their transmission to the next host e.g. strong anti-predator response and highest escape speed in infected *G. roeseli* by *P. minutus* towards non-host crustacean predator *Dikerogammarus villosus* in order prevent inappropriate transmission (Medoc and Beisel, 2008; Beisel and Médoc, 2010). Natural selection is assumed to act on traits that enables the parasite to manipulate its intermediate host's phenotype in a manner that increases the probability of trophic transmission of the parasite to the definitive host (Thomas *et al.*, 2005). One possible mechanism is manipulation of the intermediate hosts avoidance behaviour towards non-host predators in order to reduce the chance of the intermediate host by predators that are not definitive hosts, presumably increasing the likelihood of predation by the definitive host. The ultimate cause of 'increased parasite fitness through transmission to a definitive host-predator' occurs in combination with proximate causes of the success or failure of infected intermediate hosts, such as avoidance of non-host predators.

During the test and control experiments the level of oxygen in aquaria was not monitored. The treatment with the fish cues would likely have had lower levels of oxygen. It could affect the gammarids decision to whether select areas with fish chemical cues that contains low dissolved oxygen or without fish chemical cues that includes high level of dissolved oxygen.

Conclusion and recommendation

Our study revealed that parasites are capable of increasing non-host predator avoidance in *G. fossarum*. However, no effects in

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non-host predator avoidance was observed in uninfected or infected *G. pulex* and *E. berilloni*. Other studies have demonstrated modulation of host behaviour by *P. minutus* specifically in sympatric gammarids. For example, a more pronounced change in geotaxis was shown in native *G. pulex* infected by *P. minutus* compared to an infected invasive host, *G. roeselii* (Bauer *et al.*, 2005). *P. minutus* appears able to exploit the plasticity of sympatric intermediate host antipredator responses to its own advantage. Accordingly, care should be taken to ensure that species of host and species of parasite are not confounded, especially since one intermediate host species (*G. pulex*) was sampled from another river than the remaining intermediate host species (*G. fossarum* and *E. berilloni*).

Zittel *et al.* (2018) showed that the three gammarids in this study are intermediate host to a cryptic *P. minutus* 'species'. Accordingly, Zittel *et al.* (2018) recommended genetic identification of *P. minutus,* in order to ascertain if differences in observations may be attributed to different *P. minutus* species. Indeed, intermediate host species with different *P. minutus* species have different non-host predator avoidance behaviour. In other words, invasive intermediate hosts may introduce invasive parasites into the native intermediate host population possibly resulting in native parasite maladaptation in invasive new host species (Moret *et al.*, 2007). However, it is questionable if the 'increased host ability hypothesis' would vary on the cryptic species of the parasite. Water fowl is a vector for *P. minutus* between native and invasive gammarids in the Paderborn Plateau. The lower susceptibility to non-final host predators of infected *G. fossarum* could facilitate invasion by *G. fossarum* into the upper parts of the streams, and hence aid in the establishment of *G. fossarum* and non-native *P. minutus* in the ecological niche currently occupied by native gammarids by competitive exclusion. Our results may have implications in terms of the role of parasites in the success or failure of invasive species to colonize new areas.

Recent studies showed that behavioural plasticity (individual differences in behaviour) and consistency (consistent among-individual variation in behaviour) of intermediate hosts differed among both host sex and infection status (Park and Sparkes, 2017). Unfortunately, we were unable to test sex-specific behaviour of amphipods in our study, because we did not have the time to identify the sexes of individuals. We conclude that uninfected gammarids did not appear to actively avoid non-host predators. We recommend to conduct experiments with different non-host predators and host predators in order to assess differences in predators' efficiency and coevolution history of the prey–predator.

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Data availability statement. The R-code and Stan-code is available at GitHub (https://github.com/idopen/Gammarid_predator_avoidance).

Conflict of interest. None.

Ethical standards. Not applicable.

References

Abjornsson K, Dahl J, Nyström P and Brönmark C (2000) Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Journal of Experimental Marine Biology and Ecology* **34**, 379–387.

Alexander H, Thrall PH, Antonovics J, Jarosz AM and Oudemans PV (1996) Population dynamics and genetics of plant disease: a case study of anther-smut disease. Published by: Ecological Society of America. *Ecology* **77**, 990–996.

Bakker TCM, Mazzi D and Zala SM (1997) Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology* **78**, 1098–1104.

Bakker TCM, Frommen JG and Thünken T (2017) Adaptive parasitic manipulation as exemplified by acanthocephalans. *Ethology* **123**, 779–784.

Baldauf SA, Thünken T, Frommen JG, Bakker TCM, Heupel O and Kullmann H (2007) Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *International Journal for Parasitology* **37**, 61–65.

Bauer A, Haine ER, Perrot-Minnot MJ and Rigaud T (2005) The acanthocephalan parasite *Polymorphus minutus* alters the geotactic and clinging behaviours of two sympatric amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeseli*. *Journal of Zoology* **267**, 39–43.

Beisel JN and Médoc V (2010) Bird and amphipod parasites illustrate a gradient from adaptation to exaptation in complex life cycle. *Ethology Ecology and Evolution* **22**, 265–270.

Blossey B and Nötzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* **83**, 887–889.

Bürkner P (2016) brms: an R package for Bayesian multilevel models using Stan. Journal of Statistical Software.

Cézilly F and Perrot-Minnot MJ (2005) Studying adaptive changes in the behaviour of infected hosts: a long and winding road. *Behavioural Processes* **68**, 223–228.

Cézilly F and Perrot-Minnot MJ (2010) Interpreting multidimensionality in parasite-induced phenotypic alterations: panselectionism versus parsimony. *Oikos* **119**, 1224–1229.

Cezilly F, Favrat A and Perrot-Minnot M-J (2013) Multidimensionality in parasite-induced phenotypic alterations: ultimate versus proximate aspects. *Journal of Experimental Biology* **216**, 27–35.

Chivers DP, Brown GE and Smith RJF (1996) The evolution of chemical alarm signals: attracting predators benefits alarm signal senders. *The American Naturalist* **148**, 649.

Combes C (2005) Manipulations: variations on the themes of signalling and exaptation. *Behavioural Processes* **68**, 211–213.

Dezfuli BS and Giari L (1999) Amphipod intermediate host of *Polymorphus minutus*, parasite of water birds, with notes on ultrastructure of host-parasite interface. *Folia Parasitologica* **46**, 117–122.

Dezfuli BS, Maynard BJ and Wellnitz TA (2003) Activity levels and predator detection by amphipods infected with an acanthocephalan parasite, *Pomphorhynchus laevis*. *Folia Parasitologica* **50**, 129–134.

Dicke M and Grostal P (2001) Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics* **32**, 1–23.

Dodson SI, Crowl TA, Peckarsky BL, Kats LB, Covich AP and Culp JM (1994) Non-visual communication in freshwater benthos: an overview. *Journal of the North American Benthological Society* **13**, 268–282.

Friman VP, Lindstedt C, Hiltunen T, Laakso J and Mappes J (2009) Predation on multiple trophic levels shapes the evolution of pathogen virulence. *PLoS ONE* **4**, 8–13.

Gelman A, Rubin DB, Gelman A and Rubin DB (1992) Inference from iterative simulation using multiple sequences linked references are available on JSTOR for this article: inference from iterative simulation using multiple sequences. *Statistical Science* **7**, 457–472.

Haddaway NR, Vieille D, Mortimer RJG, Christmas M and Dunn AM (2014) Aquatic macroinvertebrate responses to native and non-native predators. *Knowledge and Management of Aquatic Ecosystems* **415**, 1–12. doi: 10.1051/kmae/2014036

Hettyey A, Zsarnóczai S, Vincze K, Hoi H and Laurila A (2010) Interactions between the information content of different chemical cues affect induced defences in tadpoles. *Oikos* **119**, 1814–1822.

Holomuzki JR, Short TM, Holomuzki JR, Short TM and Morgan TH (1988) Nordic society oikos habitat use and fish avoidance behaviors by the stream-dwelling isopod *Lirceus fontinalis*. *Oikos* **52**, 79–86.

Jacquin L, Mori Q, Pause M, Steffen M and Medoc V (2014) Non-specific manipulation of gammarid behaviour by *P. minutus* parasite enhances their predation by definitive bird hosts. *PLoS ONE* **9**, 1–11.

Jermacz Ł, Dzierżyńska-Białończyk A and Kobak J (2017) Predator diet, origin or both? Factors determining responses of omnivorous amphipods to predation cues. *Hydrobiologia* **785**, 173–184.

https://doi.org/10.1017/S0031182020002140 Published online by Cambridge University Press

Kaldonski N, Perrot-Minnot MJ and Cézilly F (2007) Differential influence of two acanthocephalan parasites on the antipredator behaviour of their common intermediate host. *Animal Behaviour* **74**, 1311–1317.

Kaldonski N, Perrot-Minnot MJ, Motreuil S and Cézilly F (2008) Infection with acanthocephalans increases the vulnerability of *Gammarus pulex* (Crustacea, Amphipoda) to non-host invertebrate predators. *Parasitology* **135**, 627–632.

Kats LB and Dill LM (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* **5**, 361–394.

Keane RM and Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **17**, 164–170.

MacNeil C, Dick JTA, Hatcher MJ and Dunn AM (2003) Differential drift and parasitism in invading and native *Gammarus* spp. *Crustacea: Amphipoda). Ecography* **26**, 467–473.

Matz C and Kjelleberg S (2005) Off the hook – How bacteria survive protozoan grazing. *Trends in Microbiology* **13**, 302–307.

Medoc V and Beisel JN (2008) An acanthocephalan parasite boosts the escape performance of its intermediate host facing non-host predators. *Parasitology* **135**, 977–984.

Médoc V, Rigaud T, Bollache L and Beisel JN (2009) A manipulative parasite increasing an antipredator response decreases its vulnerability to a nonhost predator. *Animal Behaviour* **77**, 1235–1241.

Médoc V, Albert H and Spataro T (2015) Functional response comparisons among freshwater amphipods: ratio-dependence and higher predation for *Gammarus pulex* compared to the non-natives *Dikerogammarus villosus* and *Echinogammarus berilloni*. *Biological Invasions* **17**, 3625–3637.

Moret Y, Bollache L, Wattier R and Rigaud T (2007) Is the host or the parasite the most locally adapted in an amphipod-acanthocephalan relationship? A case study in a biological invasion context. *International Journal for Parasitology* **37**, 637–644.

Müller J (2000) Mitochondrial DNA variation and the evolutionary history of cryptic *Gammarus fossarum* Types. *Molecular Phylogenetics and Evolution* **15**, 260–268.

Nickol BB (1985) Epizootiology. In Crompton DWT and Nickol BB (eds), *Biology of the Acanthocephala*. Cambridge: Cambridge University Press, pp. 307–346.

Park T and Sparkes TC (2017) Multidimensionality of modification in an isopod-acanthocephalan system. *Frontiers in Ecology and Evolution* **5**, 1–13.

Parker IM and Gilbert GS (2004) The evolutionary ecology of novel plant-pathogen interactions. *Annual Review of Ecology, Evolution, and Systematics* **35**, 675–700.

Perrot-Minnot MJ, Kaldonski N and Cézilly F (2007) Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod. *International Journal for Parasitology* **37**, 645–651.

Perrot-Minnot MJ, Maddaleno M and Cézilly F (2016) Parasite-induced inversion of geotaxis in a freshwater amphipod: a role for anaerobic metabolism? *Functional Ecology* **30**, 780–788.

Pöckl S, Building A, Drive R, House TF and Sawrey F (2003) Life history and reproductive capacity of *Gammarus fossarum* and *G. roeseli* (Crustacea: Amphipoda) under naturally fluctuating water temperatures: a simulation study. *Freshwater Biology* **48**, 53–66.

Prenter J, MacNeil C, Dick JTA and Dunn AM (2004) Roles of parasites in animal invasions. *Trends in Ecology and Evolution* **19**, 385–390.

R Core Team, R (2018) A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rigby MC and Jokela J (2000) Predator avoidance and immune defence: costs and trade-offs in snails. *Proceedings of the Royal Society B: Biological Sciences* **267**, 171–176.

Rohde K (1994) Niche restriction in parasites: proximate and ultimate causes. *Parasitology* **109**, S69–S84.

R Studio Team (2018) R studio: integrated development for R. RStudio, Inc., Boston. Available at http://www.rstudio.com/.

Schmidt-Drewello A, Riss HW, Scharsack JP and Meyer EI (2016) Relative benefit of the invasive *Echinogammarus berilloni* (Catta, 1878) over native gammarids under fish predation (*Gasterosteus aculeatus* Linnaeus, 1758). *Aquatic Ecology* **50**, 75–85.

Siegismund HR and Müller J (1991) Genetic structure of *Gammarus fossarum* populations. *Heredity* **66**, 419–436.

Smith LA and Webster MM (2015) *Gammarus pulex* show a grouping response to conspecific injury cues but not to predator kairomones. *Behavioral Ecology* **26**, 1188–1195.

Spencer EE, Newsome TM and Dickman CR (2017) Prey selection and dietary flexibility of three species of mammalian predator during an irruption of non-cyclic prey. *Royal Society Open Science* **4**, 170317. doi: 10.1098/rsos.170317

Stan Development Team (2018) Stan modeling language users guide and reference manual. Version 2.18.0. Available at http://mc-stan.org/.

Stone CF and Moore J (2014) Parasite-induced alteration of odour responses in an amphipod-acanthocephalan system. *International Journal for Parasitology* **44**, 969–975.

Szokoli F, Winkelmann C, Berendonk TU and Worischka S (2015) The effects of fish kairomones and food availability on the predator avoidance behaviour of *Gammarus pulex*. *Fundamental and Applied Limnology/Archiv für Hydrobiologie* **186**, 249–258.

Tain L, Perrot-Minnot M-J and Cezilly F (2006) Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proceedings of the Royal Society B: Biological Sciences* **273**, 3039–3045.

Thomas F, Adamo S and Moore J (2005) Parasitic manipulation: where are we and where should we go? *Behavioural Processes* **68**, 185–199.

Truhlar AM and Aldridge DC (2015) Differences in behavioural traits between two potentially invasive amphipods, *Dikerogammarus villosus* and *Gammarus pulex*. *Biological Invasions* **17**, 1569–1579.

Van Buskirk J, Krügel A, Kunz J, Miss F and Stamm A (2014) The rate of degradation of chemical cues indicating predation risk: an experiment and review. *Ethology* **120**, 942–949.

Ward AJW (2012) Social facilitation of exporation in mosquito fish (*Gambusia Holbrooki*). *Behavioral Ecology and Sociobiology* **66**, 223–230.

Watanabe S (2010) Asymptotic equivalence of Bayes cross validation and widely applicable information criterion in singular learning theory. *Journal of Machine Learning Research* **11**, 3571–3594.

Wilhelm FM and Schindler DW (2011) Effects of *Gammarus lacustris* (Crustacea: Amphipoda) on plankton community structure in an alpine lake. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1401–1408.

Wisenden BD, Cline A and Sparkes TC (1999) Survival benefit to antipredator behaviour in the amphipod *Gammarus minus* (Crustacea: Amphipoda) in response to injury released chemical cues from conspecifics and heterospecifics. *Ethology* **105**, 407–414.

Wudkevich K, Wisenden BD, Chivers DP and Smith RJF (1997) Reactions of *Gammarus lacustris* to chemical stimuli from natural predators and injured conspecifics. *Journal of Chemical Ecology* **23**, 1163–1173.

Zittel M, Grabner D, Wlecklik A, Sures B, Leese F, Taraschewski H and Weigand AM (2018) Cryptic species and their utilization of indigenous and non-indigenous intermediate hosts in the acanthocephalan *Polymorphus minutus* sensu *lato* (Polymorphidae). *Parasitology* **145(11)**, 1–9. doi: 10.1017/S0031182018000173

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