

Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours

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Abstract

Many parasites with complex life cycles increase the chances of reaching a final host by adapting strategies to manipulate their intermediate host's appearance, condition or behaviour. The acanthocephalan parasite *Pomphorhynchus laevis* uses freshwater amphipods as intermediate hosts before reaching sexual maturity in predatory fish. We performed a series of choice experiments with infected and uninfected *Gammarus pulex* in order to distinguish between the effects of visual and olfactory predator cues on parasite-induced changes in host behaviour. When both visual and olfactory cues, as well as only olfactory cues were offered, infected and uninfected *G. pulex* showed significantly different preferences for the predator or the non-predator side. Uninfected individuals significantly avoided predator odours while infected individuals significantly preferred the side with predator odours. When only visual contact with a predator was allowed, infected and uninfected gammarids behaved similarly and had no significant preference. Thus, we believe we show for the first time that *P. laevis* increases its chance to reach a final host by olfactory-triggered manipulation of the anti-predator behaviour of its intermediate host.

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1. Introduction

The colonisation of new host individuals is one of the most critical phases in a parasite's life cycle. In cases where this cycle includes intermediate hosts, it is often necessary that the intermediate host is ingested by the parasite's final host (trophic transmission). This creates a strong selective pressure on the parasite to increase the probability that its intermediate host is eaten by the final host (Lafferty, 1999 and citations within; Moore, 2002).

There are two ways in which a parasite can reach this goal. Firstly, it could make the intermediate host more conspicuous to predators (Bakker et al., 1997). A striking example is the digenean parasite *Leucochloridium macrostomum*, that turns the eye stalks of the freshwater snail

Succinea putris into colourful blinker lamps, making the snails an easy prey for water ouzels (Gibson et al., 2002). Second, it could alter the intermediate host's behaviour (Holmes and Bethel, 1972). One of the best known examples of this kind of manipulation is the small liver fluke *Dicrocoelium dendriticum*. Cercariae of this parasite manipulate the subpharyngeal ganglion of their intermediate host, *Formicaria* ants. An infected ant will not return to its nest but instead locks its mandibula onto the top of blades of grass, thus raising the probability that the parasite will be ingested by grazing sheep, the parasite's final host (Mehlhorn, 2001).

Acanthocephala represent another well-known group of manipulative parasites that infect arthropods, like isopods and amphipods, as intermediate hosts and vertebrates, especially fishes and birds, as definitive hosts (Crompton and Nickol, 1985). Cystacanths of the genera *Pomphorhynchus* and *Polymorphus* are known to change the photopho-

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bic behaviour of their amphipod intermediate hosts. While uninfected individuals prefer to hide in dark places, infected amphipods will move toward a light source (Bethel and Holmes, 1973; Bakker et al., 1997). *Polymorphus minutus* alters the geotactic and clinging behaviour of two sympatric *Gammarus* species, the native *G. pulex* as well as the invasive *G. roeseli* (Bauer et al., 2005). *Pomphorhynchus laevis* increases the activity level of *Echinogammarus stammeri*, resulting in a higher representation of infected individuals in the water column during the day (Maynard et al., 1998). This should result in a higher probability for the parasite to complete its life cycle, because non-hiding and hyperactive intermediate hosts are more prone to fish predation (Crowden and Broom, 1980; Krause and Godin, 1994; Lafferty and Morris, 1996; Berdoy et al., 2000; Thomas et al., 2002; Mazzi and Bakker, 2003; Médouc et al., 2006).

Alternative hypotheses exist for behavioural changes associated with parasitism (see Thomas et al., 2005 and citations therein). For example, increased host activity might consist of increased foraging movements due to higher energy requirements (Milinski, 1985). If host behaviour is manipulated by the parasite, one would expect an increase in fitness (transmission) of the parasite at the expense of the host's fitness. One approach to test this prediction is to investigate host behaviour in the presence of predators (Dahl et al., 1998). Uninfected *Gammarus* sp. usually avoid predators. If infected individuals are attracted to predators, this would strongly support the manipulation hypothesis. Clear evidence for parasite manipulation was found by Hechtel et al. (1993) examining the anti-predator behaviour of the isopod *Caecidotea intermedius* infected with *Acanthocephalus dirus*. In side-choice experiments, where *C. intermedius* could choose between a predator and a non-predator side of a test tank, infected individuals spent more time on the predator's side of the tank, whereas uninfected individuals avoided the predator's side (Hechtel et al., 1993). Further experiments on crustaceans using predatory odour stimuli only showed significant differences between parasitized and non-parasitized individuals in anti-predator behaviour; they did not demonstrate a preference for predators in infected individuals (Jakobsen and Wedekind, 1998; Dezfuli et al., 2003; Wellnitz et al., 2003).

The aim of our study was to distinguish between the effects of visual and olfactory predator cues on parasite-induced changes in intermediate host behaviour by the acanthocephalan parasite *P. laevis*. *Gammarus pulex* served as intermediate hosts, and *Perca fluviatilis* L. served as definitive hosts. Side choice experiments were based on the setup of Hechtel et al. (1993).

2. Materials and methods

2.1. Experimental subjects

Several hundred infected and uninfected *G. pulex* were collected in April 2002 from the brook Waldibach near

Lucerne and transferred to the laboratory at the Eawag, Kastanienbaum, Switzerland. They were kept in two flow-channels (3.20 × 0.45 × 0.18 m) with constant water flow from Lake Lucerne under natural light and temperature conditions (light/dark: 16/8 h, 11 °C). The channels were equipped with dead leaves and algae to provide nutrition and shelter. The infection status of gammarids could be determined visually, because the orange cystacanth in the dorsal coelom is visible through the cuticle. Each morning, the gammarids were sorted according to their infection status and transferred to separate basins (35 × 20 × 25 cm) for use in the trials that day. All amphipods were used only once.

Perch were used as predators in all experiments, because they are known to include *Gammarus* sp. in their diet (Kelleher et al., 1998). We used 10 sub-adult perch measuring approximately 10 cm, which were caught from Lake Lucerne using minnow traps. They were kept in a plastic tank (60 × 40 × 23 cm) equipped with several shelters, a layer of sand, leaf litter and constant water flow from Lake Lucerne. Several times a day, perch were fed uninfected *G. pulex* ad libitum. No individual perch was used in two consecutive trials. At the end of the experiments all perch were released in Lake Lucerne.

2.2. Experimental design

All experiments were conducted between 20 April and 5 May 2002. In the first experiment, gammarids were exposed to visual and olfactory predator cues. Choice tests were conducted in a plastic tank measuring 25 × 15 × 15 cm. The tank was divided into an upper and a lower section using a transparent green net placed 3 cm above the bottom (mesh size 2 mm; Fig. 1). The net lay on transparent plastic spacers which were glued to the tank. The upper section was divided into two equal-sized compartments using a removable, opaque, dark-grey plastic partition.

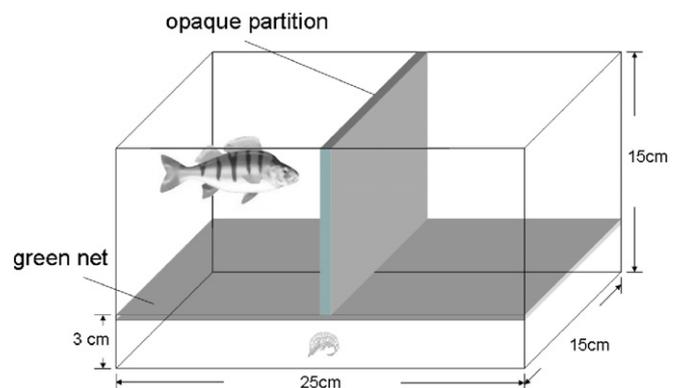


Fig. 1. Set-up of the test tank in the experiments with olfactory and visual cues, and with olfactory cues only. Perch or perch-conditioned water was placed randomly in the left or right compartment. The gammarid was able to choose between the predator and non-predator side by using the full horizontal space.

In each trial we placed an infected or an uninfected *G. pulex* in the middle of the lower section where it could move freely to each side. We then installed the net and the plastic partition. After an acclimatisation time of 5 min, we placed a perch in one of the two upper compartments, using a net for transport between the perch basin and the tank. In order to exclude tank side biases, the position of the perch was alternated between trials.

One minute after the introduction of the stimulus, we started to record the position of the *G. pulex* by observations from above. For a period of 5 min we observed, at 1 min intervals, whether the *G. pulex* was on the fish or the non-fish side of the tank. Thus we recorded five positions for each individual. In all trials the observer was unaware of the infection status of the *G. pulex*.

In the second experiment gammarids were exposed to only visual cues. We put the tank from the first experiment into an unmodified tank (25 × 15 × 15 cm), thereby creating a lower chamber for the gammarids that extended an additional 3 cm below the experimental tank. Because the bottom of the upper tank was transparent, gammarids in the lower chamber were exposed to visual, but not olfactory, predator cues. The deployment of fish and observations were similar to the first experiment.

In the third experiment we exposed gammarids to only olfactory cues. The tank setup complied with the setup from the first experiment. Instead of a fish, we simultaneously added 100 ml of perch-conditioned water into one compartment and as a control 100 ml of unconditioned lake water into the other. Perch-conditioned water was taken from the perch tank immediately before the start of the experiment. To exclude tank side biases, the compartment containing the perch-conditioned water was alternated between trials. Observations were made in a manner similar to the other experiments.

In all experiments the tanks were surrounded by white styropore to avoid disturbances from outside. The set-up was illuminated by two fluorescent tubes (35 W) placed 1.6 m above the tank. After the experiments, the body length of all gammarids was determined to the nearest mm using digital calipers. After each test, the water in the tank was completely replenished.

As an additional control for tank side bias we ran another test series without adding any predator cues. Neither uninfected nor infected *G. pulex* significantly preferred a particular side of the test tank (Wilcoxon test, $N = 13$, $z = -0.5$, $P = 0.617$, and $N = 15$, $z = -0.115$, $P = 0.908$, respectively). Thus side effects can be excluded.

2.3. Statistics

Non-parametric statistics were applied due to the ordinal nature of the data. Given test probabilities are two-tailed. P values <0.05 were considered statistically significant. Analyses were performed using SPSS 11.0.1 statistical package.

3. Results

When combined visual and olfactory predator cues were presented, there was a significant difference in the sides chosen by uninfected and infected gammarids (Mann–Whitney U test: $N_{\text{uninfected}} = 17$, $N_{\text{infected}} = 15$, $z = -3.78$, $P < 0.0001$; Fig. 2). While uninfected individuals significantly preferred the non-fish side of the test tank (Wilcoxon test: $N = 17$, $z = -3.56$, $P < 0.001$; Fig. 2), infected individuals tended to stay on the fish side (Wilcoxon test: $N = 15$, $z = -1.65$, $P = 0.099$; Fig. 2).

When there were only visual cues present, uninfected and infected individuals had similar side preferences (Mann–Whitney U test: $N_{\text{uninfected}} = 21$, $N_{\text{infected}} = 20$, $z = -0.274$, $P = 0.784$; Fig. 2). Neither uninfected (Wilcoxon test: $N = 21$, $z = -0.241$, $P = 0.784$; Fig. 2) nor infected (Wilcoxon test: $N = 20$, $z = -0.459$, $P = 0.646$; Fig. 2) gammarids preferred or avoided the fish side.

When only olfactory predator cues were given, the choice of uninfected and infected gammarids differed significantly (Mann–Whitney U test: $N_{\text{uninfected}} = 19$, $N_{\text{infected}} = 18$, $z = -3.992$, $P < 0.0001$; Fig. 2). Uninfected individuals showed a significant preference for the non-fish side (Wilcoxon test: $N = 19$, $z = 3.1$, $P = 0.002$; Fig. 2), while infected gammarids significantly preferred the side in which perch-conditioned water was added (Wilcoxon test: $N = 18$, $z = -2.759$, $P = 0.006$; Fig. 2).

Uninfected gammarids were significantly larger than infected ones in each experiment (Mann–Whitney U test: all $z < -3.297$, all $P < 0.001$). However, body size did not significantly correlate with the strength of preference of uninfected or infected individuals in each experiment (Spearman rank correlation: all r between -0.266 and 0.213 , all $P > 0.388$).

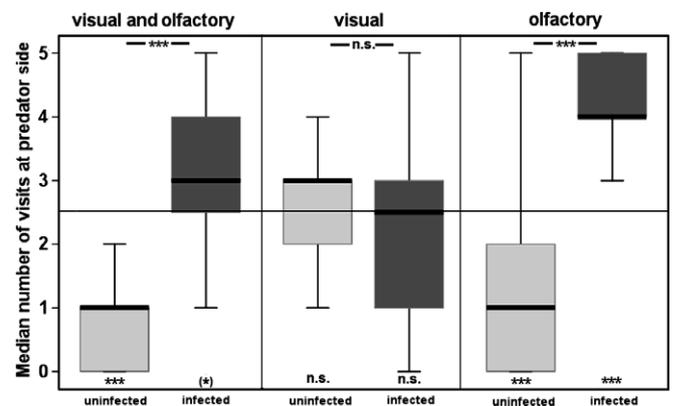


Fig. 2. Median (\pm quartiles, percentiles) number of visits from uninfected (bright bars) and infected (dark bars) gammarids at the predator's side when combined visual and olfactory cues, only visual cues, or only olfactory cues were given. Differences in choice behaviour between infected and uninfected gammarids were tested with Mann–Whitney U tests (upper statistics); while deviations from random choice were tested with Wilcoxon tests (bottom statistics). *** $P < 0.001$; (*) $P < 0.1$; n.s. = $P > 0.1$.

4. Discussion

Gammarus pulex infected with *P. laevis* showed a different response towards predators than uninfected individuals. When exposed to combined visual and chemical cues as well as to chemical cues alone, uninfected individuals avoided the predator side, whereas infected individuals were attracted to it. Visual cues alone induced no significant side preference in either group.

Behavioural differences in presence of predatory stimuli between parasitized and non-parasitized intermediate host individuals were reported in several species (Hechtel et al., 1993; Bakker et al., 1997; Jakobsen and Wedekind, 1998; Dezfuli et al., 2003 and references therein; Wellnitz et al., 2003). A change of host behaviour due to infection could be the consequence of two different mechanisms. Firstly, an infection could have indirect effects on the host system, e.g. lowering a host's condition by parasitizing its metabolism as a side effect of pathology (Milinski, 1985). Second, the parasite could directly manipulate its host's behaviour (Moore, 2002). Both mechanisms ultimately result in increased transmission of the parasite, but in the first case the change in host behaviour is a side effect of adaptive host response, whereas in the second it is controlled by the parasite's genome. In our experiments, infected gammarids showed a preference for the predator's odour, whereas uninfected ones strongly avoided it, suggesting that *P. laevis* affects its gammarid host's behaviour in order to enhance the likelihood of transmission to the final host.

Hechtel et al. (1993) showed that *A. dirus* manipulates the behaviour of its intermediate host, the isopod *C. intermedius* by reversing its anti-predator behaviour. However, this study did not distinguish between different predatory cues. The odour of a predator altered the activity level of uninfected, but not of infected, *E. stammeri* (Dezfuli et al., 2003; Wellnitz et al., 2003). Uninfected *Macrocyclops albidus* copepods adjusted their behaviour to olfactory cues in order to reduce predation risk while individuals infected with procercoids of *Schistocephalus solidus* tended to increase predation risk (Jakobsen and Wedekind, 1998). Our study showed that olfactory cues alone were sufficient to induce anti-predator behaviour in uninfected individuals and a manipulated pro-predator response in infected gammarids. Manipulation of olfactory perception by a parasite has also been shown in other host–parasite systems like *Rattus norvegicus*–*Toxoplasma gondii*. Here *T. gondii* alters the perception of cat predation risk in its intermediate host, a rat, by turning the rat's aversion to cat odour into attraction (Berdoy et al., 2000).

Infected gammarids showed a significant preference for olfactory fish cues, but counter-intuitively this preference was weaker when both visual and olfactory information were available. This could be a result of different concentrations of olfactory cues in the experiments. The perch-conditioned water was an odour mix of 10 individuals and therefore probably more concentrated. Furthermore,

in the first experiment with live fish, one infected individual totally avoided the predator, possibly because of an early infection state. Due to the fact that we did not determine the maturity of the cystacanth, this is just a hypothetical guess. Excluding this individual from the presented results, the sample yielded a significant preference (Wilcoxon test: $N = 14$, $z = -2.28$, $P = 0.023$) for the predator side.

Infected individuals were significantly smaller than uninfected ones. This effect could have resulted from a reduced growth due to parasitisation or high predation pressure on large, infected individuals. However, body size did not correlate with the strength of predator preference in uninfected or infected individuals. Additionally, Bauer et al. (2005) report no effect of body size on phototactic behaviour in uninfected and infected *G. pulex*.

Visual information of a predatory fish alone does not seem to play a decisive role in initiating escape behaviour in gammarids. Former experiments showed phototactic behaviour in gammarids (Bethel and Holmes, 1973). Thus *G. pulex* should be able to discriminate between light and dark. However, the results indicate that gammarids cannot visually detect predators by their moving shadows. The distance to the predator was, however, somewhat larger in this experiment.

In *G. pulex*, chemical cues seem to be the most important factors when recognising predators. We cannot distinguish whether fish odour or decomposition products of eaten *Gammarus* sp. triggered the observed behaviour. In summary, we believe we show for the first time that a parasite increases its chance to reach a final host by olfactory-triggered manipulation of the anti-predator behaviour of its intermediate host.

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