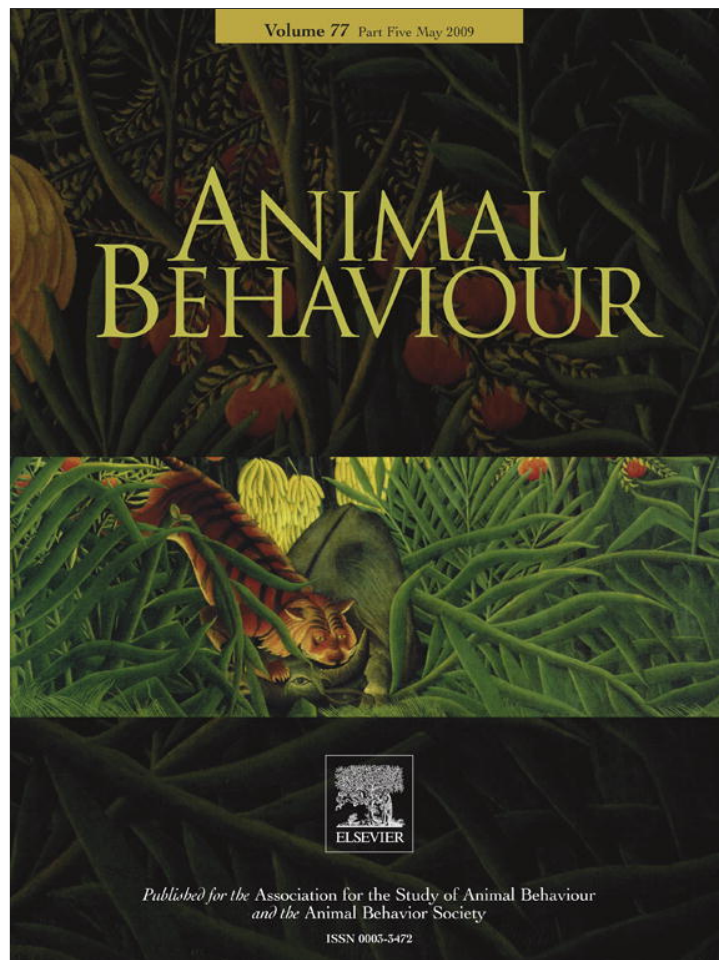


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/yanbe

A manipulative parasite increasing an antipredator response decreases its vulnerability to a nonhost predator

Vincent Médoc^{a,*}, Thierry Rigaud^{b,1}, Loïc Bollache^{b,1}, Jean-Nicolas Beisel^a

^aLaboratoire des Interactions Ecotoxicologie, Biodiversité, Ecosystèmes (LIEBE), UMR CNRS 7146, Université Paul Verlaine-Metz

^bEquipe Ecologie Evolutive, Laboratoire Biogéosciences, UMR CNRS 5561, Université de Bourgogne

ARTICLE INFO

Article history:

Received 29 September 2008
Initial acceptance 28 November 2008
Final acceptance 24 January 2009
Published online 18 March 2009
MS. number: 08-00633

Keywords:

antipredator response
behavioural manipulation
Gammarus roeseli
nonhost avoidance
olfactory cue
Polymorphus minutus
refuge usage

Trophically transmitted parasites have to deal with the antipredator adaptations of their intermediate hosts. Some of these parasites induce behavioural changes in their intermediate hosts that make them more vulnerable to predation by definitive hosts. However, the adaptiveness of behavioural manipulation also depends on the probability of being eaten by a nonhost predator. Parasites might therefore try to use specific antipredator responses of intermediate hosts to avoid this dead end. We tested this hypothesis using the acanthocephalan *Polymorphus minutus* and its intermediate amphipod host, *Gammarus roeseli*. In their natural habitat, uninfected *G. roeseli* shelter near the river bottom while infected gammarids tend to cling onto floating materials. In microcosm experiments, infected amphipods were preyed upon by sticklebacks (nonhost predators) less than uninfected individuals when refuges were provided near the water surface. Regardless of their infection status, *G. roeseli* reacted to olfactory cues of predation by spending more time on refuges and near the surface. However, these behaviours were increased in infected gammarids. These results suggest that, in addition to specific induced behaviours, parasites may also enhance an existing antipredator behaviour to increase their own survival.

© 2009 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Trophically transmitted parasites rely on intermediate hosts to reach their definitive hosts and complete their life cycle (Poulin 1998; Combes 2001). Numerous parasite species are known to alter the phenotype of their intermediate hosts. Among the various changes induced by infection, those that increase parasites' transmission efficiency are considered to be evolutionarily selected adaptations (Moore 2002; Thomas et al. 2005). Previous studies have reported enhanced transmission rates when the manipulated host is selectively preyed upon by a suitable definitive host (Bethel & Holmes 1977; Lyndon 1996; Bakker et al. 1997; Fuller et al. 2003; Lagrue et al. 2007). However, alterations of the intermediate host's phenotype potentially lead to increased predation by a large range of predators, including those that cannot be successfully infected by the parasite (i.e. 'nontarget' predators). Consequently, to be considered adaptive, manipulation should show some degree of specificity by predisposing infected hosts more strongly to predation by the next hosts (i.e. 'target' predators) than to inappropriate

ones (Cézilly & Perrot-Minnot 2005). Seppälä & Jokela (2008), however, demonstrated that even nonspecific changes in behaviour might be adaptive if target hosts are the most common predators in the parasite's natural environment. The remaining question is whether manipulative parasites may evolve nonhost avoidance strategies when the costs associated with predation risk by nonhosts are too high to be compensated for by nonspecific manipulation. Only a few empirical studies have convincingly demonstrated that changes in infected host phenotypes could lead to a decrease in the vulnerability to predation by inappropriate hosts (Levri 1998; Médoc et al. 2006). On the other hand, a number of studies have shown that modified behaviours increase the susceptibility of intermediate hosts to nonhost predators (Ness & Foster 1999; Mouritsen & Poulin 2003; Kaldonski et al. 2008) or, at best, make no difference to predation probability by unsuitable hosts (Seppälä et al. 2006; Lagrue et al. 2007).

Prey species have evolved numerous defence mechanisms to reduce their susceptibility to predation (reviewed in Edmunds 1974; Sih 1987; Abrams 2001). The chemical detection of predatory species is widespread among aquatic organisms and allows a high level of specificity (Kats & Dill 1998; Wisenden 2000). Olfactory cues appear to be a stimulus for various defence mechanisms including behavioural responses, such as reduced activity and increased use of shelters (Lima & Dill 1990). Thus, parasites using

* Correspondence: V. Médoc, Laboratoire des Interactions Ecotoxicologie, Biodiversité, Ecosystèmes (LIEBE), UMR CNRS 7146, Université Paul Verlaine-Metz, Campus Bridoux, avenue du Général Delestraint, 57070 Metz-Borny, France.

E-mail address: medoc@univ-metz.fr (V. Médoc).

¹ T. Rigaud and L. Bollache are at the Equipe Ecologie Evolutive, Laboratoire Biogéosciences, UMR CNRS 5561, Université de Bourgogne, 2100 Dijon, France.

prey species to reach their definitive host via trophic transmission have to deal with such predator assessment by the host. Some parasites manipulating their host behaviour can modify how hosts perceive predators, infected prey sometimes being attracted by the predator instead of being repulsed (Berdoy et al. 2000; Perrot-Minnot et al. 2007). However, no studies to date have shown that infected prey use the perception of a nonhost predator and the antipredator behaviour that follows to avoid inappropriate hosts.

Now naturalized in France, *Gammarus roeseli* is a freshwater amphipod of Balkan origin that became widespread in Western Europe over the past century (Karaman & Pinkster 1977; Jazdzewski 1980; Barnard & Barnard 1983). Under predation risk, this crustacean decreases its activity and shelters near the river bottom while its dorsal spines reduce the capture efficiency of fish (Bollache et al. 2006). In natural populations, *G. roeseli* is commonly infected by the acanthocephalan parasite *Polymorphus minutus*, and infected gammarids are known to swim close to the water surface and cling onto floating materials. This behaviour is thought to favour the parasite's transmission to its definitive host, a waterbird (Cézilly et al. 2000; Bauer et al. 2005). *Polymorphus minutus* has been used in three previous studies on predation by nondefinitive hosts, making it one of the best-known parasites in this respect. Médoc et al. (2006) showed that the presence of a benthic arthropod predator increases the amount of time that *P. minutus*-infected *G. roeseli* spend near the water surface, consequently decreasing the predation rate by this nonhost predator. Using *Gammarus pulex*, another intermediate host of *P. minutus*, Kaldonski et al. (2007) found that an antipredator reaction (increasing the level of refuge usage) was as strong in infected animals as in uninfected ones when the predator was a benthic fish (*Cottus gobio*). However, they did not find any decrease in predation associated with this behaviour. In addition, Kaldonski et al. (2008) found differences in the predation rates by invertebrate predators, but the link to refuge usage was unclear. These studies suggest that gammarids infected by *P. minutus* may be able to detect and escape predators inappropriate for the parasite. The inconsistencies between escape behaviours and differential predation noted by Kaldonski et al. (2007, 2008) could be because refuges provided to gammarids in these experiments did not allow infected individuals to express the modified behaviour fully (i.e. swimming at the top of the water column and clinging to floating refuges), since the refuges provided were limited to the benthic area (Kaldonski et al. 2007, 2008).

Our aim in our study was to test the ability of *G. roeseli* infected by *P. minutus*, compared to uninfected individuals, to perceive predation risk by fish and escape predation in different refuge conditions. We first determined the prevalence of infection in the main natural habitats of *G. roeseli*. We then measured predation preference of a nonhost predator in microcosm experiments. The three-spined stickleback, *Gasterosteus aculeatus*, was chosen as a predator because it lives in sympatry with *G. roeseli* in the study site and amphipods are known to be part of its diet (Hynes 1950; Hart 2003). Its foraging behaviour is also well documented (Hart & Ison 1991; Gill & Hart 1999). Finally, as *G. roeseli* commonly uses chemical communication to assess predation risk (Baumgärtner et al. 2003; Bollache et al. 2006), we investigated how *P. minutus*-infected *G. roeseli* perceived and reacted to olfactory cues. That is, we recorded behaviour of amphipods exposed to water scented or not by sticklebacks.

METHODS

Study Species and Sampling

We studied the *G. roeseli* population living in the Nied river, Laquenexy, northeastern France (49°05'N, 6°19'E). *Gammarus roeseli* is the only amphipod species present and is parasitized by *P. minutus*, the only macroparasite of *G. roeseli* found at this site.

To assess accurately the distribution of infected gammarids at this site, we investigated the prevalence of *P. minutus* at the mesohabitat scale. In June 2005, a 100 m long representative section of the stream was defined to identify the natural habitat that covered more than 5% of the river bottom. This preliminary step allowed us to select six distinct natural habitats for the study. Invertebrates were collected with a Surber sampler from these substrates, including floating organic materials (FOM), *Nuphar lutea* leaves, the dominant hydrophyte *Potamogeton pectinatus*, willow roots, pebbles (diameter: 6–8 cm) and stones (diameter: 15–20 cm). These organic and mineral substrates differed in their location along the water column, ranging from the water surface (FOM and *N. lutea*, 0–15 cm depth) to the river bottom (pebbles and stones, 40–50 cm depth), with the hydrophytes and roots being located between these extremes (20–30 cm depth). We sampled each habitat three times and sorted invertebrates in the laboratory, using a binocular magnifying glass (Leica MZ 125). Dissection of amphipods followed to confirm infection by *P. minutus*.

For experimental purpose, amphipods and fish were collected at the same site in 2006, with a pond net (500 µm mesh). The presence of *P. minutus* cystacanths observed through the cuticle of infected *G. roeseli* distinguished them from uninfected individuals. To remove potential host sex or parasitic-load effects, only male amphipods harbouring one cystacanth were used in the experiments. Males were identified during the precopula mate-guarding phase to avoid any confusion between the two genders. Selected gammarids measured approximately 7 mm in length to match the buccal aperture of sticklebacks and allow prey ingestion (following pre-experimental investigations). Selected fish were males of equal size (65 ± 3 mm in length) showing a red breeding coloration.

Housing

During transport to the laboratory, gammarids and fish were maintained, respectively, in 3 litre and 30 litre opaque plastic tanks, at a density that did not exceed 50 individuals/litre for *G. roeseli* and 4 individuals/litre for *G. aculeatus*. We placed some plants collected from the river (*P. pectinatus*) in each tank to reduce the stress induced by handling and transport.

In the laboratory, amphipods were maintained separately from sticklebacks in plastic tanks (33 × 25 cm and 13 cm high) at a density of 20 individuals/litre. Sticklebacks were placed in larger tanks (60 × 29 cm and 31 cm high) at a density of 2 individuals/litre. Each housing unit was filled with filtered site water and we added some natural plants (*P. pectinatus*) to reduce the risk of death from cannibalism for gammarids, and to minimize aggression between males for fish. Oxygen was supplied by a filter pump which also generated a flow inside the tanks. Animals were housed in a thermoregulated room to ensure a stable water temperature (16 ± 1 °C) and with a 12:12 h light:dark period.

The experimental subjects were maintained under these laboratory conditions for 8 days before experiments began. During this time, gammarids were fed at satiation with Alder-sheet discs while sticklebacks received live chironomids as food (20 chironomids per fish per day). Sticklebacks were deprived of food for 24 h before experiments to standardize the predation pressure among the experimental units. All specimens were used only once. At the end of the experiments, gammarids were dissected to confirm their infection status while surviving sticklebacks were released in the Nied river, in accordance with European legislation. Nine of the 80 sticklebacks collected for the study died in captivity for unknown reasons. Approval for the study was granted by the Ministère de l'Écologie et du Développement Durable.

Vulnerability to Predation

Twenty *G. roeseli* (10 uninfected and 10 infected) were placed in opaque aquaria (33 × 25 cm and 13 cm high) partially filled with 8 litres of filtered water drawn at the site. Proper oxygenation was ensured by an air pump fixed on one corner and six discs (diameter: 18 mm) of conditioned Alder-sheets were distributed to invertebrates as a nutritive resource. Aquaria were placed inside a wide vat (140 × 110 cm and 40 cm high) partially filled with water and acting as a 'water bath' system. A pump ensured water circulation and a cooler (Huber TC40E) allowed a high degree of thermal stability (16 ± 1 °C). Water temperature was checked regularly. The device was illuminated by four light tubes (Philips, 36 W) mounted 60 cm above the water surface; the light:dark period was 12:12 h.

To provide shelters from fish predation and prevent cannibalism, 40 glass pebbles (diameter: 15 mm; four white, 18 green and 18 brown) were haphazardly placed on the bottom of each aquarium, convex side down. In addition to this benthic refuge, since gammarids are known to use plants as refuges to avoid predation (Kaldonski et al. 2007), a dense artificial plant (33 × 8 cm and 7 cm high) was added in some experimental series. Artificial plants were preferred to natural plants to avoid a potential confounding effect of plant usage for feeding. We tested three situations (experimental groups): (1) with glass pebbles only (control), (2) with glass pebbles and the artificial plant placed horizontally on the bottom of the aquarium (gammarids allowed to use refuges only at the bottom), (3) with glass pebbles and the artificial plant placed vertically in the aquarium, from bottom to surface (amphipods allowed to use refuges from the bottom to the water surface). Each situation was replicated 20 times (21 for the group with no plant) both with *G. roeseli* alone (mortality control groups) and in the presence of *G. aculeatus* (experimental groups).

In experimental groups, after an acclimatizing period of 1 h, one stickleback was added in each aquarium. After pilot experiments, we chose an exposure time of 24 h without a complex refuge and 48 h in the presence of artificial plants. At the end of the experiments, the fish were removed and the surviving *G. roeseli* were counted.

Differential predation on uninfected and *P. minutus*-infected *G. roeseli* by sticklebacks was assessed using Manly's alpha (preference index, Manly 1974; Chesson 1978), which allows for the depletion of prey during the course of the experiment. Replicates with no predation and those with fewer than four amphipods consumed were removed from analyses. The preference index was calculated using the equation:

$$\alpha_i = \frac{\ln p_i}{\sum_{j=1}^m p_j}$$

where α_i is the Manly's index for prey type *I* (here infected prey), p_i and p_j the proportion of prey *i* or *j* remaining at the end of the trial and *m* is the number of prey types. The index ranges from 0 to 1 (when only uninfected or infected prey were consumed, respectively). Observed values were compared with a situation of absence of preference ($\alpha_i = 0.5$).

Gammarids' Response to Predation Cues

We equipped a Pyrex crystallizing dish (diameter: 140 mm; height: 74 mm) with eight equidistant plastic strips acting as artificial refuges (height: 60 mm; width: 10 mm; depth: 3 mm). In contrast to glass, the plastic strips were porous to allow amphipods to cling to them. We used a silicone glue to fix the strips vertically onto the side wall of the dish, from bottom to water surface. As the strips came up to the water surface, amphipods were allowed to

cling onto refuges along the water column. The dish was filled with 900 ml of water from the collecting site, oxygenated and temperature stabilized (16 ± 1 °C) at least 48 h prior to experiments. The water was either free of any olfactory cue (controls) or scented by sticklebacks during a predation event. The scented water was obtained from the previous experiments, using the replicates in which the fish ate 10 prey (five uninfected and five infected specimens, representing 1.25 predation events per litre) after a 24 h exposure. This process was expected to provide a predation signal close to the one observed under natural conditions, because it includes both the fish odour and the chemical cues released by injured gammarids (Wudkevich et al. 1997; Abjörnsson et al. 2000; Wisenden et al. 2001). To protect the organisms from any external disturbance, we performed tests in a closed box (28 × 28 cm and 37 cm high) equipped with a source of diffuse light and a webcam (Philips ToUcam Pro II Pcv840) to film the horizontal plan. Filming began 5 min after a single *G. roeseli* was introduced into the device. The recording time was 3 min at a rate of 20 frames/s. Experiments were replicated 20 times with both uninfected and *P. minutus*-infected gammarids. The video shots were analysed frame by frame with an unpublished software developed at the LIEBE laboratory (designed by Philippe Rousselle). We first measured the time spent in contact with refuges, during which gammarids remained static, clinging onto the strip, or moved slightly along it. Then, we calculated the proportion of refuge time spent clinging at the surface. Finally, we measured the time spent close to the surface, including swimming and sheltering periods.

Statistical Analyses

Differences in the prevalence of *P. minutus* between habitats were analysed using a nested ANOVA design (after arcsine square-root transformation of the data), where depth category was a fixed factor, and the substrates were random factors nested within depth category, followed by a Tukey honestly significant difference (HSD) post hoc test.

In predation experiments, we first compared the general predation rate (proportion of gammarids, regardless of their infection status, eaten by the fish) between the three experimental groups, using an ANOVA on arcsine square-root-transformed data, followed by a Dunnett's test (Quinn & Keough 2002), to assess differences between the control group (with no plants) and each group where plants were added. We then compared within groups the observed Manly's alpha with a situation of equal predation vulnerability ($\alpha_i = 0.5$) using paired *t* tests. These data met normality (Shapiro-Wilk *W* tests: all $P > 0.19$) so we used parametric statistics. The difference between groups in α_i (i.e. according to refuge availability) was assessed using an ANOVA, followed by a Dunnett's test (control group: without plants).

The effects of parasitism and olfactory cue on the behaviour of *G. roeseli* were tested by two-way ANOVAs. Data for the proportion of time spent clinging onto refuges at the surface, and the time spent at the surface, were transformed (arcsine square-root and Box-Cox transformations, respectively) to meet normality and homogeneity of variance assumptions.

We performed all tests with a 5% type I error risk, using STATISTICA 6.0 (Statsoft, Tulsa, OK, U.S.A.) and JMP 6.0 (SAS Institute Inc., Cary, NC, U.S.A.) software. All tests were two tailed.

RESULTS

Amphipod Distribution

Of the 3431 *G. roeseli* collected, we identified 455 *P. minutus*-infected specimens (13.2% prevalence). The prevalence differed

between sampling depths, while variation between substrates within each depth category was not significant (ANOVA on arcsine square-root-transformed data: global model: $F_{5,12} = 10.76$, $r^2 = 0.82$, $P = 0.0004$; effect of the fixed factor depth: $F = 26.30$, $P = 0.01$; effect of the nested random factor substrate: $F = 0.97$, $P = 0.44$; Fig. 1). A Tukey HSD post hoc test revealed that prevalence of *P. minutus* was higher in hosts collected in near-surface habitats than in deeper habitats (Fig. 1).

Vulnerability to Predation

No mortality was observed in control groups (without *G. aculeatus*), regardless of infection and refuge location, suggesting that *G. roeseli* were not cannibalistic during the experiments. In experimental units, the average general predation rates by *G. aculeatus* on *G. roeseli* \pm SEM were 0.59 ± 0.05 ($N = 21$) when no plants were added to the aquaria, 0.49 ± 0.06 ($N = 20$) when plants were added at the bottom, and 0.40 ± 0.05 ($N = 20$) when the plant refuges reached the water surface (ANOVA: $F_{2,60} = 3.24$, $P = 0.04$). Only the group where plants were at the surface differed significantly from the group without plants (Dunnett's test for comparison with a control group: $P = 0.33$ for plants on the bottom; $P = 0.02$ for plants at the surface). Hence, an artificial plant reaching the surface significantly reduced the general predation rate, even after a longer exposure (48 h versus 24 h).

With no plant in the aquarium, sticklebacks showed no predation preference according to prey infection status (Fig. 2). When plant-like refuges were added at the bottom, sticklebacks ate slightly fewer infected amphipods than uninfected ones. The difference in predation was more marked when plants reached the surface (Fig. 2) and varied between groups (ANOVA: $F_{2,48} = 3.31$, $P = 0.04$). The difference from the control group (without plants) was not significant for series where plants were on the bottom of aquaria (Dunnett's: $P = 0.18$), but was significant for aquaria equipped with plants reaching the water surface (Dunnett's: $P = 0.03$).

Refuge Usage Under Stressful Conditions

Infection with *P. minutus* had a significant effect on *G. roeseli* behaviour, whatever the olfactory cue conditions (Table 1, Fig. 3). Infected gammarids spent more time on refuges than uninfected

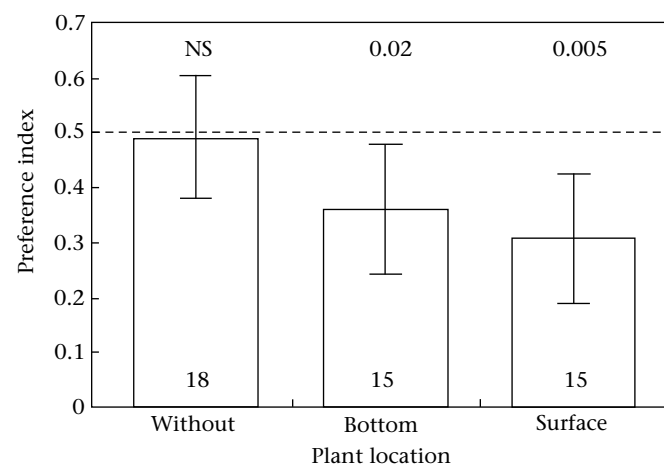


Figure 2. Differential predation (*Polymorphus minutus*-infected gammarids versus uninfected specimens) of *Gammarus roeseli* interacting with nonhost fish, estimated using Manly's α_i , as a function of refuge location (artificial plant). Means are given \pm 95% confidence intervals. Values below the dashed line indicate underconsumption of infected prey. Numbers in the bars are sample sizes; numbers above the bars are P values for comparisons within groups of the observed values with no preference ($\alpha_i = 0.5$).

ones (Fig. 3a) and, during this sheltering time, spent more time clinging in the near-surface area (Fig. 3b). Considering the swimming and the sheltering periods together, infected amphipods spent more time at the surface than uninfected ones, the latter remaining mainly benthic (Fig. 3c).

Chemical cues related to predation by *G. aculeatus* (fish odour in addition to chemical signals released by injured gammarids) also significantly increased the time spent on refuges and the time spent at the surface for both uninfected and *P. minutus*-infected gammarids (Table 1, Fig. 3a, c). However, olfactory cues did not significantly alter the clinging behaviour of *G. roeseli* during sheltering time (Table 1, Fig. 3b).

Finally, interactions between parasitism and olfactory cue were never significant (Table 1), so changes in the behaviour of *G. roeseli* induced by olfactory cues did not differ with infection status.

Table 1

Results of two-way ANOVA testing for differences in the time spent in refuges, the proportion of time clinging onto refuges at the surface and the time spent at the surface (either swimming or clinging onto refuges) among *Gammarus roeseli*, in relation to infection by *Polymorphus minutus* and olfactory cues related to the predatory activity of three-spined sticklebacks

Source	df	Sum of squares	F	P
Time spent on refuges				
Parasitism	1	14034.27	13.244	<0.001
Olfactory cue	1	21081.10	19.894	<0.001
Parasitism*olfactory cue	1	756.71	0.714	0.401
Error	78	82655.56		
Proportion of refuge time at surface				
Parasitism	1	3.52	28.367	<0.001
Olfactory cue	1	0.42	3.395	0.069
Parasitism*olfactory cue	1	0.01	0.055	0.815
Error	78	9.68		
Time spent at surface				
Parasitism	1	16689.37	30.752	<0.001
Olfactory cue	1	3010.58	5.547	0.021
Parasitism*olfactory cue	1	116.17	0.214	0.645
Error	78	42331.20		

Prior to analysis, data for the proportion of time spent clinging onto refuges at the surface and the time spent at the surface were transformed (arcsine square-root and Box-Cox transformations, respectively. See text for details and Fig. 3 for differences between infected and uninfected animals).

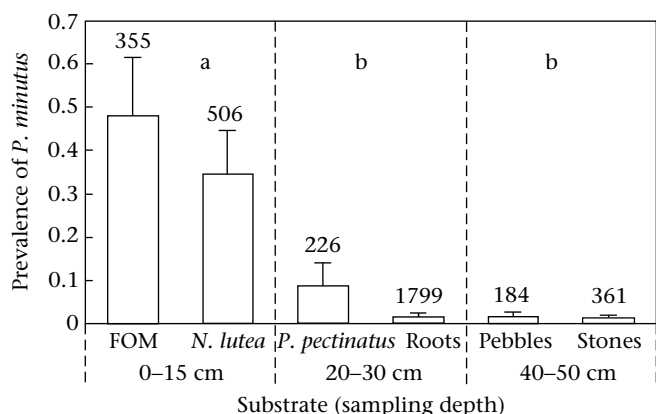


Figure 1. Prevalence of *Polymorphus minutus* in the freshwater amphipod *Gammarus roeseli*. Six distinct natural habitats were sampled in the Nied river (northeastern France), including organic (floating organic materials = FOM, *Nuphar lutea*, *Potamogeton pectinatus* and willow roots) and mineral substrates (pebbles and stones). The samples also differed in their position along the water column (sampling depth). $N = 3$ replicates and means are given \pm SEM. The numbers above the bars are gammarid sample sizes. Depth categories with different letters showed significantly different prevalences after Tukey HSD post hoc tests ($P \leq 0.05$).

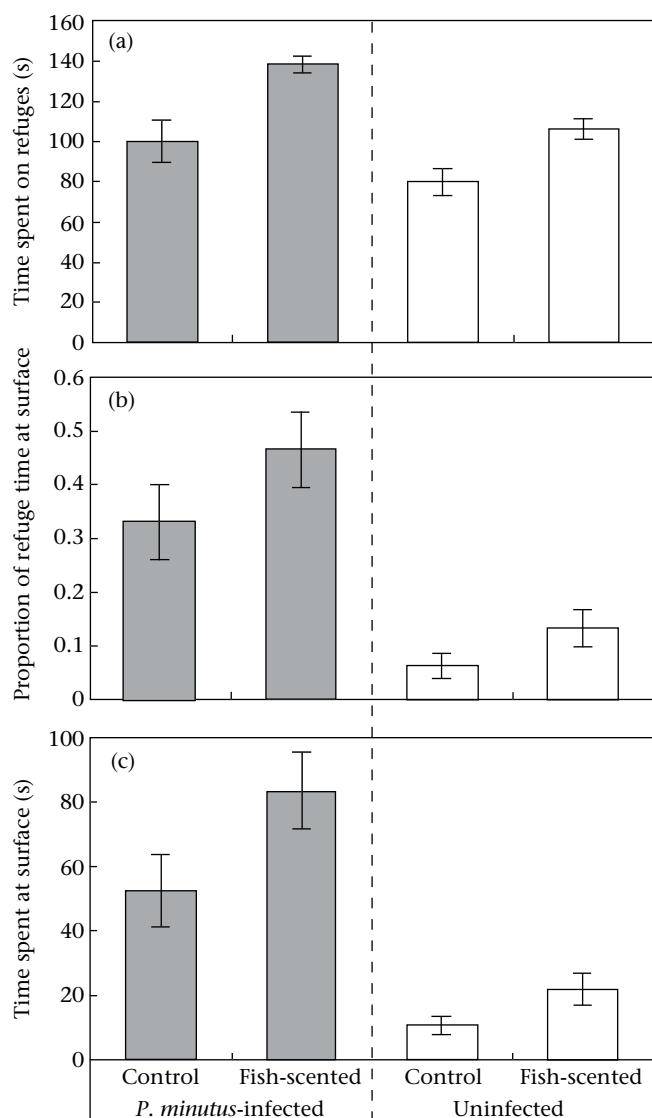


Figure 3. Refuge use by *Gammarus roeseli* infected with *Polymorphus minutus* under stressful conditions. (a) The time spent at refuges, (b) the proportion of time clinging onto refuges at the surface and (c) the time spent at the surface (swimming and clinging onto refuges) were obtained for infected (grey bars) and uninfected (white bars) amphipods using control or predator-scented water. Means are given \pm SEM. See Table 1 for the ANOVA results.

DISCUSSION

Decreased Vulnerability to a Nonhost Predator

Overall, our results suggest that infection with this acanthocephalan parasite can decrease the vulnerability of amphipods to a nonhost predator. *Gammarus roeseli* infected by *P. minutus* were significantly less preyed upon by sticklebacks than uninfected amphipods, mostly in experiments with refuges which allowed the hosts to express the full behavioural modification induced by the parasite (i.e. to reach the water surface and cling to a refuge).

We showed, in accordance with results of previous studies (MacNeil et al. 2003; Wellnitz et al. 2003), that acanthocephalan parasites structure their intermediate host population at a micro-spatial scale, both in nature and in our experiments. *Gammarus roeseli* infected by *P. minutus* spent most of their time clinging onto refuges available at the water surface, confirming the results of

Bauer et al. (2005). These results were all obtained on naturally infected gammarids, and it might be argued that gammarids behave differently for reasons unrelated to parasitism, or that the abnormal behaviour is the cause and not the consequence of infection. This is improbable, however, both because not all infections of *G. roeseli* by acanthocephalans are linked to behavioural alterations (Moret et al. 2007) and because recent experimental infections using acanthocephalans showed that the abnormal behaviours were induced only after the host became infected (Franceschi et al. 2008). It might also be argued that *P. minutus* infection does not modify *G. roeseli*'s perception of water depth (i.e. reverse geotaxis), but rather modifies its habitat choice, gammarids showing a high affinity for floating materials once infected. This is not the case because in a previous study performed on the same host population, we observed the same spatial distribution (infected specimens located near the water surface) with the use of artificial traps (Médoc et al. 2006). These traps were distributed along the water column so as to provide a standard mesohabitat that spanned the full range of depth categories.

We found a strong congruence between the results from predation experiments and those from behavioural experiments (both refuge usage and response to a predation risk cue). The reversed geotaxis and increased refuge usage of infected amphipods explained why they were less exposed to the predation by sticklebacks than uninfected prey, especially when they were allowed to shelter in the near-surface area. Kaldonski et al. (2007), studying the consequences of *P. minutus* infection on *G. pulex*, also found an increase in refuge usage when predator chemical cues were present. However, it did not translate to a difference in predation risk. The main difference from our experiments is that the shelters provided to gammarids were not present throughout the water column, therefore preventing infected gammarids from expressing their modified behaviour fully, as they could in our study where plants were fixed at the bottom. This therefore suggests that the whole sequence of the manipulated behaviour is necessary for the host to escape inappropriate predators of the parasite. When no refuges were provided, sticklebacks indiscriminately ate the two prey types. Again, this suggests that a single component of behavioural change (increasing the time spent at the surface) is not enough to escape the fish predator. This also suggests that differences in appearance linked with the infection did not modify the stickleback's preference for one prey type. Uninfected and infected gammarids differed in appearance because cystacanths are visible as orange dots through the host's cuticle. This result contrasts with the observations reported by Bakker et al. (1997) who found that the coloration of acanthocephalans is an effective signal increasing the conspicuousness of infected prey, and therefore contributes to the increased trophic transmission of parasites. Bakker et al. (1997) nevertheless worked with fish acanthocephalans, which could explain the different results from ours, but it appears that a visual signal from the parasites as a component of parasite-induced phenotype manipulation is not a general pattern.

Does Enhancement of Existing Behaviour Explain Manipulated Behaviour?

An increase in refuge usage under chemical stimulation is an antipredator behaviour commonly reported among gammarids (*G. lacustris*: Wudkevich et al. 1997; *G. minus*: Wisenden et al. 1999; *G. pulex*: Baldauf et al. 2007; Perrot-Minnot et al. 2007). Here, we showed that this behaviour also exists in *G. roeseli* (see also Bollache et al. 2006). *Polymorphus minutus*-infected as well as uninfected individuals perceived the olfactory cue related to the predation by sticklebacks and reacted to it by increasing their

sheltering time. Kaldonski et al. (2007) reported a similar increase in refuge usage in *P. minutus*-infected *G. pulex* when exposed to another nonhost fish, the bullhead, *Cottus gobio*. Whether the predator signal was present or absent in our study, sheltering time was higher in infected *G. roeseli* than in uninfected ones.

In addition to an enhanced refuge usage, *P. minutus* infection was also associated with an increased time spent at the surface. This raises an interesting question about adaptive sophistication: can we consider negative geotaxis and refuge usage as two distinct manipulations driven by two distinct selective forces (target and nontarget hosts, respectively)? Two hypotheses can be advanced: (1) the weak hypothesis, where both negative geotaxis and refuge usage have been selected to maximize transmission to birds, the reduced predation by fishes being a fortunate side-effect and (2) the strong hypothesis, where the parasite expresses two distinct adaptive manipulations, the first increasing the encounter probability between infected prey and target hosts (negative geotaxis), and the second decreasing their vulnerability to predation by nontarget hosts (refuge usage). The results of the predation experiment tend to be consistent with the strong hypothesis. Indeed, following the weak hypothesis, infected gammarids would be expected to reach the top of the water column and then to stop in surface refuges to be easily preyed upon by birds. Consequently, infected gammarids should have experienced a higher predation rate than uninfected conspecifics when the artificial plant was placed on the bottom of the experimental unit. This was not the case, suggesting that one of the modified behaviours (e.g. refuge usage) can be expressed independently of the others (e.g. negative geotaxis).

Taken together, our findings suggest that parasitic manipulation can be an enhancement of existing antipredator behaviours. To be precise, we do not know whether the time spent at the surface is an efficient antipredator behaviour (this requires at least further investigations). However, both increased sheltering behaviour and increased time spent at the surface were, on the one hand, a reaction to a predator cue, and on the other hand, considerably increased by the infection by *P. minutus*. Therefore, we propose that the behavioural alterations induced by this parasite enhance natural behavioural responses to predators, pre-existing in their host. The *P. minutus* behavioural manipulation could therefore consist of enhancing an antipredator behaviour in its intermediate host, a situation contrasting with some other parasite-induced behavioural changes. Indeed, several studies performed on fish acanthocephalans found hyperactive intermediate hosts (Maynard et al. 1998; Dezfuli et al. 2003), sometimes attracted to fish odours (Baldauf et al. 2007; Perrot-Minnot et al. 2007). Perrot-Minnot et al. (2007) described how 'reverse antipredator behaviours' enhance the trophic transmission of an acanthocephalan parasite to an appropriate fish host. Here, our results suggest that enhanced antipredator behaviours may allow avoidance of a species that cannot be successfully infected by the parasite.

The fact that intermediate hosts become more efficient in facing certain predators once infected may nevertheless appear counter-intuitive when prey organisms are expected to do all they can anyway to avoid being eaten. One explanation can be provided that considers life history budgeting in manipulated hosts. Indeed, *P. minutus* is known to decrease the pairing success in *G. pulex* significantly while infected females are totally castrated (Bollache et al. 2001, 2002). In the same way, infection with the other fish acanthocephalan *Echinorhynchus truttae* significantly decreases the number of isopods (*Asellus aquaticus*) killed by *G. pulex* (Fielding et al. 2003). Trophically transmitted parasites, which have little interest in these host behaviours (sexual and foraging activities), shift the balance towards antipredator behaviours that specifically reduce predation risk by nonhosts.

In addition to enhanced refuge usage and reversed geotaxis (negative instead of positive), infected *G. roeseli* also showed clinging behaviour, which consists of sheltering close to the water surface. This *P. minutus*-induced change appeared more specific to infected individuals than the other two changes because it was significantly related to parasitism, but not to the olfactory cue. This argues for the multidimensionality of host manipulation by *P. minutus*, demonstrating that parasites modify more than one dimension in the phenotype of their hosts (Cézilly & Perrot-Minnot 2005). This idea is also supported by the recent finding that *P. minutus*-infected *G. roeseli* interacting with a crustacean predator have a higher escape speed than uninfected individuals (Médoc & Beisel 2008). Such a behaviour, which seems a priori inconsistent with an increased trophic transmission to host predators, could nevertheless be of selective importance for *P. minutus* if it reduces predation risk by nonhosts. As pointed out by Cézilly & Perrot-Minnot (2005), one remaining question to be answered is how these different behavioural alterations are linked (e.g. by a common mechanistic basis).

To conclude, although the transmission of *P. minutus*-infected *G. roeseli* to waterbirds remains to be quantified (data concerning increased transmission to birds relate to another *Polymorphus* species, see Bethel & Holmes 1977), our results provide evidence for a parasitic manipulation preventing ingestion by a nonhost predator, therefore showing some degree of specificity.

Acknowledgments

This study was supported by the French Ministère de l'Ecologie et du Développement Durable and funded by a grant from the Conseil Régional de Lorraine. We thank Clément Lagrue for constructive comments, Philippe Rouselle for software conception and Maxime Mehl, Pascal Sornom and Clément Bojic for help during laboratory and field work. We are grateful to two anonymous referees for their valuable comments on the paper.

References

- Abjörnsson, K., Dahl, J., Nyström, P. & Brönmark, C. 2000. Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Aquatic Ecology*, **34**, 379–387.
- Abrams, P. A. 2001. Predator – prey interactions. In: *Evolutionary Ecology: Concepts and Case Studies* (Ed. by C. W. Fox, D. A. Roff & D. J. Fairbairn), pp. 277–289. Oxford: Oxford University Press.
- Bakker, T. C. M., Mazzi, D. & Zala, S. 1997. Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology*, **78**, 1098–1104.
- Baldauf, S. A., Thünken, T., Frommen, J. G., Bakker, T. C. M., Heupl, O. & 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.
- Barnard, J. L. & Barnard, C. M. 1983. *Freshwater Amphipoda of the World*. Virginia: Hayfield Associates.
- Bauer, A., Haine, E. R., Perrot-Minnot, M.-J. & 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.
- Baumgärtner, D., Koch, U. & Rothhaupt, K. O. 2003. Alteration of kairomone-induced antipredator response of the freshwater amphipod *Gammarus roeseli* by sediment type. *Journal of Chemical Ecology*, **29**, 1391–1401.
- Berdoy, M., Webster, J. P. & Macdonald, D. W. 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. *Proceedings of the Royal Society of London, Series B*, **267**, 1591–1594.
- Bethel, W. M. & Holmes, J. C. 1977. Increased vulnerability of amphipods to predation owing to altered behavior induced by larval acanthocephalans. *Canadian Journal of Zoology*, **55**, 110–115.
- Bollache, L., Gambade, G. & Cézilly, F. 2001. The effects of two acanthocephalan parasites, *Pomphorhynchus laevis* and *Polymorphus minutus*, on pairing success in male *Gammarus pulex* (Crustacea: Amphipoda). *Behavioral Ecology and Sociobiology*, **49**, 296–303.
- Bollache, L., Rigaud, T. & Cézilly, F. 2002. Effects of two acanthocephalan parasites on the fecundity and pairing status of female *Gammarus pulex* (Crustacea: Amphipoda). *Journal of Invertebrate Pathology*, **79**, 102–110.

- Bollache, L., Kaldonski, N., Troussard, J. P., Lagrue, C. & Rigaud, T. 2006. Spines and behaviour as defences against fish predators in an invasive freshwater amphipod. *Animal Behaviour*, **72**, 627–633.
- Cézilly, F. & Perrot-Minnot, M. J. 2005. Studying adaptive changes in the behaviour of infected hosts: a long and winding road. *Behavioural Processes*, **68**, 223–228.
- Cézilly, F., Grégoire, A. & Bertin, A. 2000. Conflict between co-occurring manipulative parasites? An experimental study of the joint influence of two acanthocephalan parasites on the behaviour of *Gammarus pulex*. *Parasitology*, **120**, 625–630.
- Chesson, J. 1978. Measuring preference in selective predation. *Ecology*, **59**, 211–215.
- Combes, C. 2001. *Parasitism: the Ecology and Evolution of Intimate Interactions*. Chicago: University of Chicago Press.
- Dezfuli, B. S., Maynard, B. J. & Wellnitz, T. A. 2003. Activity level and predator detection by amphipods infected with an acanthocephalan parasite, *Pomphorhynchus laevis*. *Folia Parasitologica*, **50**, 129–134.
- Edmunds, M. 1974. *Defence in Animals. A Survey of Anti-Predator Defences*. Harlow: Longman.
- Fielding, N. J., MacNeil, C., Dick, J. T. A., Elwood, R. W., Riddell, G. E. & Dunn, A. M. 2003. Effects of the acanthocephalan parasite *Echinorhynchus truttae* on the feeding ecology of *Gammarus pulex* (Crustacea: Amphipoda). *Journal of Zoology*, **26**, 321–325.
- Franceschi, N., Bauer, A., Bollache, L. & Rigaud, T. 2008. The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. *International Journal for Parasitology*, **38**, 1161–1170.
- Fuller, C. A., Rock, P. & Phillips, T. 2003. Behavior, color changes, and predation risk induced by acanthocephalan parasitism in the Caribbean termite *Nasutitermes acajutlae*. *Caribbean Journal of Science*, **39**, 128–135.
- Gill, A. B. & Hart, P. J. B. 1999. Dynamic changes in prey choice by stickleback during simultaneous encounter with large prey. *Journal of Fish Biology*, **55**, 1317–1327.
- Hart, P. J. B. 2003. Habitat use and feeding behaviour in two closely related fish species, the three-spined and nine-spined stickleback: an experimental analysis. *Ecology*, **72**, 777–783.
- Hart, P. J. B. & Ison, S. 1991. The influence of prey size and abundance, and individual phenotype on prey choice by the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology*, **40**, 205–218.
- Hynes, H. B. N. 1950. The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of methods used in studies of the food of fishes. *Journal of Animal Ecology*, **19**, 36–58.
- Jazdzewski, K. 1980. Range extensions of some gammaridean species in European inland waters caused by human activity. *Crustaceana, Supplement (Leiden)*, **6**, 84–107.
- Kaldonski, N., Perrot-Minnot, M.-J. & 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, M.-J., Motreuil, S. & Cézilly, F. 2008. Infection with acanthocephalans increases the vulnerability of *Gammarus pulex* (Crustacea, Amphipoda) to non-host invertebrate predators. *Parasitology*, **135**, 627–632.
- Karaman, G. S. & Pinkster, S. 1977. Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea-Amphipoda) Part II. *Gammarus roeseli*-group and related species. *Bijdragen tot de Dierkunde*, **47**, 165–196.
- Kats, L. B. & Dill, L. M. 1998. The scent of death, chemosensory assessment of predation risk by prey animals. *Ecoscience*, **5**, 361–394.
- Lagrue, C., Kaldonski, N., Perrot-Minnot, M.-J., Motreuil, S. & Bollache, L. 2007. Modification of hosts' behavior by a parasite: field evidence for adaptive manipulation. *Ecology*, **88**, 2839–2847.
- Levri, E. P. 1998. Perceived predation risk, parasitism, and the foraging behavior of a freshwater snail (*Potamopyrgus antipodarum*). *Canadian Journal of Zoology*, **76**, 1878–1884.
- Lima, S. L. & Dill, L. M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.
- Lyndon, A. R. 1996. The role of acanthocephalan parasites in the predation of freshwater isopods by fish. In: *Aquatic Predators and their Preys* (Ed. by S. P. R. Greenstreet & M. L. Tasker), pp. 27–32. London: Fishing News Books.
- MacNeil, C., Fielding, N. J., Hume, K. D., Dick, J. T. A., Elwood, R. W., Hatcher, M. J. & Dunn, A. M. 2003. Parasite altered micro-distribution of *Gammarus pulex* (Crustacea: Amphipoda). *International Journal for Parasitology*, **33**, 57–64.
- Manly, B. F. J. 1974. A model for certain types of selection experiments. *Biometrics*, **30**, 281–294.
- Maynard, B. J., Wellnitz, T. A., Zanini, N., Wright, W. G. & Dezfuli, B. S. 1998. Parasite-altered behavior in a crustacean intermediate host: field and laboratory studies. *Journal of Parasitology*, **84**, 1102–1106.
- Médoc, V. & Beisel, J.-N. 2008. An acanthocephalan parasite boosts the escape performance of its intermediate host facing non-host predators. *Parasitology*, **135**, 1–8.
- Médoc, V., Bollache, L. & Beisel, J.-N. 2006. Host manipulation of a freshwater crustacean (*Gammarus roeseli*) by an acanthocephalan parasite (*Polymorphus minutus*) in a biological invasion context. *International Journal for Parasitology*, **36**, 1351–1358.
- Moore, J. 2002. *Parasites and the Behavior of Animals*. Oxford: Oxford University Press.
- Moret, Y., Bollache, L., Wattier, R. & 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.
- Mouritsen, K. M. & Poulin, R. 2003. Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator's nightmare. *International Journal for Parasitology*, **33**, 1043–1050.
- Ness, J. H. & Foster, S. A. 1999. Parasite-associated phenotype modifications in threespine stickleback. *Oikos*, **85**, 127–134.
- Perrot-Minnot, M.-J., Kaldonski, N. & Cézilly, F. 2007. Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod. *International Journal for Parasitology*, **37**, 645–651.
- Poulin, R. 1998. *Evolutionary Ecology of Parasites: From Individuals to Communities*. London: Chapman & Hall.
- Quinn, G. P. & Keough, M. J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.
- Seppälä, O., Karvonen, A. & Valtonen, E. T. 2006. Susceptibility of eye fluke-infected fish to predation by bird hosts. *Parasitology*, **132**, 575–579.
- Seppälä, O. & Jokela, J. 2008. Host manipulation as a parasite transmission strategy when manipulation is exploited by non-host predators. *Biology Letters*, **4**, 663–666.
- Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. In: *Predation: Direct and Indirect Impacts on Aquatic Communities* (Ed. by W. C. Kerfoot & A. Sih), pp. 203–224. Hanover, New Hampshire: New England University Press.
- Thomas, F., Adamo, S. & Moore, J. 2005. Parasitic manipulation: where are we and where should we go? *Behavioural Processes*, **68**, 185–199.
- Wellnitz, T., Giari, L., Maynard, B. & Dezfuli, B. S. 2003. A parasite spatially structures its host population. *Oikos*, **100**, 263–268.
- Wisenden, B. D. 2000. Olfactory assessment of predation risk in the aquatic environment. *Philosophical Transactions of the Royal Society of London, Series B*, **355**, 1205–1208.
- Wisenden, B. D., Cline, A. & Sparkes, T. C. 1999. Survival benefit to antipredator behavior in the amphipod *Gammarus minus* (Crustacea: Amphipoda) in response to injury-released chemical cues from conspecifics and heterospecifics. *Ethology*, **105**, 407–414.
- Wisenden, B. D., Pohlman, S. G. & Watkin, E. E. 2001. Avoidance of conspecific injured-released chemical cues by free-ranging *Gammarus lacustris* (Crustacea: Amphipoda). *Journal of Chemical Ecology*, **27**, 1249–1258.
- Wudkevich, K., Wisenden, B. D., Chivers, D. P. & Smith, R. J. F. 1997. Reactions of *Gammarus lacustris* to chemical stimuli from natural predators and injured conspecifics. *Journal of Chemical Ecology*, **23**, 1163–1173.